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Journal canadien des sciences halieutiques et aquatiques

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Design: Ludvic Saleh & Associates, Ottawa

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 50, No. 3, March 1993 **Volume 50, nº 3, mars 1993**

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Daily Growth Rates as Indicated by Valve Ridges in Postlarval Giant Scallop (*Placopecten magellanicus*) (Bivalvia: Pectinidae)

G. Jay Parsons[1]

Department of Zoology, University of Guelph, Ont. N1G 2W1, Canada

Shawn M. C. Robinson

Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada

John C. Roff

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1, Canada

and Michael J. Dadswell

Department of Biology, Acadia University, Wolfville, N.S. B0P 1X0, Canada

Parsons, G. J., S. M. C. Robinson, J. C. Roff, and M. J. Dadswell. 1993. Daily growth rates as indicated by valve ridges in postlarval giant scallop (*Placopecten magellanicus*) (Bivalvia: Pectinidae). Can. J. Fish. Aquat. Sci. 50: 456–464.

Postlarval giant scallop (*Placopecten magellanicus*) were examined for daily growth ridges and growth rates by marking the dissoconch shell with Alizarin red dye. The surface of the left valve of postlarvae was composed of concentric ridges, each consisting of a series of irregularly shaped raised nodules. Ridges were clear and distinct in newly settled scallop between ≈0.25 and 2.0 mm shell height. The shell of postlarvae >2 mm was pigmented and ribbed and ridges were no longer distinguishable. Estimated age was significantly correlated with actual age, suggesting that growth ridges were produced daily, under the environmental conditions of Passamaquoddy Bay. Mean growth rate ranged from 32 to 57 $\mu m \cdot d^{-1}$ and was proportional to size and age, but growth of individual scallop showed no coherence in their daily growth patterns. The short-term growth ridges in postlarval giant scallop can be used to determine age and can be applied to comparative growth, mortality, and recruitment studies of newly settled individuals <2.0 mm (≈40 d old postsettlement). The high accuracy and precision of age determination for postlarval scallop differs from studies of short-term internal growth increments of bivalve shells and larval fish otoliths.

Les auteurs ont examiné des pétoncles géants (*Placopecten magellanicus*) de stade postlarvaire dans le but d'étudier les anneaux de croissance quotidiens de même que les taux de croissance par marquage de la coquille dissoconque au colorant alizarine. La surface de la coquille gauche des postlarves était formée de crêtes concentriques, elles-mêmes formées d'une série de nodules soulevés de forme irrégulière. Les crêtes étaient nettes et distinctes chez les pétoncles récemment déposées dont la hauteur de coquille se situait entre 0,25 et 2,0 mm environ. La coquille des postlarves de plus de 2 mm de hauteur était pigmentée et striée; les crêtes n'étaient plus discernables. L'âge estimé présentait une corrélation positive avec l'âge réel, ce qui porte à croire que les crêtes de croissance étaient produites à chaque jour, du moins dans les conditions environnementales de la baie Passamaquoddy. Le taux de croissance moyen variait entre 32 et 57 $\mu m \cdot d^{-1}$ et était proportionnel à la taille et à l'âge, mais la croissance de pétoncles donnés n'était pas cohérente avec leur régime de croissance quotidien. Les crêtes de croissance temporaires des pétoncles géants de stade postlarvaire permettent de déterminer l'âge et peuvent servir à des études comparatives de la croissance, de la mortalité et du recrutement des individus de moins de 2,0 mm qui se sont déposés depuis peu (âgés de 40 jours environ, après s'être déposés). La détermination très exacte et précise de l'âge des pétoncles postlarvaires diffère de celle utilisée pour les études des accroissements à court terme des parties internes des coquilles de bivalves ou des otolithes de larves de poissons.

Received June 19, 1992
Accepted September 11, 1992
(JB527)

Reçu le 19 juin 1992
Accepté le 11 septembre 1992

The ability to estimate the age of an organism is a valuable tool for providing information about its recruitment, growth, and mortality. Ageing studies, based on accretionary structures of marine invertebrates and fish, are numerous (for review, see Clark 1974; Rhoads and Lutz 1980). However, it is essential that such ageing techniques be validated by mark–recapture studies or with animals of known age because inaccurate estimates can result in erroneous interpretations of the dynamics of populations (e.g. Beamish and McFarlane 1983; Bradford 1991).

Workers examining growth marks (external ridges or internal increments) in bivalve shells have identified various growth patterns and periodicities, such as hourly, daily, every 2 d, tidal, monthly, reproductive, seasonal, and annual increments (Pannella and MacClintock 1968; Kennish 1980; Lutz and

[1]Present address: Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada.

Rhoads 1980). Near daily or daily growth lines have been identified in several species of larval and juvenile scallops, but the frequency of deposition of such structures can be influenced by age, size, and season (Antoine 1978; Broom and Mason 1978; Gruffydd 1981). Bourget et al. (1991) have questioned the caliber of evidence concerning periodic growth marks in a number of bivalve studies. They proposed that hypotheses on the frequency of growth mark formation should be rejected unless the variability in the data is accounted for and that the effects of potential sources of variation are evaluated.

The giant scallop (*Placopecten magellanicus* Gmelin, 1791) is a subtidal boreotemperate bivalve with a spawning period ranging from July to October (Bourne 1964; Parsons et al. 1992). Larvae are planktotrophic for 30–40 d, and settlement occurs at about 250 μm (Culliney 1974) from September to November (Dadswell et al. 1988). The dissoconch shell of postlarval scallop has been described by Merrill (1959, 1961) and Waller (1972). Valves are translucent and the external surface of the left (upper) valve has concentric nodules of foliated calcite. Hurley et al. (1987) have reported near daily ridges and growth rates in larval giant scallop. Annual growth marks in adults and juvenile growth rates have also been described (Stevenson and Dickie 1954; Parsons and Dadswell 1992), but no studies have tested for periodic growth ridges or examined growth rates in the postlarval stage (i.e. newly settled and metamorphosed animals) of this commercially important species.

The aim of this study was to validate the production of daily growth ridges in postlarval giant scallop using marked, known-age animals to determine if growth ridges can be used to assess growth rate. We describe growth ridge formation and shell growth rates of this early life-history stage under field conditions.

Material and Methods

Site and Spat Collection

Passamaquoddy Bay, which is located off the western side of the Bay of Fundy, New Brunswick, has semidiurnal tides with a maximum tidal range of 8.3 m and a well-mixed water column (Trites and Garrett 1983). Summer and autumn temperatures range from 8 to 12°C and salinity from 30 to 32‰.

Postlarval giant scallop were caught using artificial collectors consisting of 500 g of monofilament gillnetting material stuffed into 2-mm-mesh "onion" bags (75 × 42 cm) (Naidu and Cahill 1986; Dadswell and Parsons 1991). The collectors were tied, by SCUBA divers, to a suspended horizontal line 3 m off-bottom at an underwater aquaculture site located off Tongue Shoal, Passamaquoddy Bay (45°04′N, 67°01′W).

Marking and Ageing Study

Twelve collectors were deployed at Tongue Shoal during August 1989 for 10 d. Collectors were retrieved by encasing the bags in 253-μm-mesh plankton nets and transporting them to the laboratory in circulating seawater tanks onboard the boat. The 12 bags were held in a 388-L recirculating tank containing 0.5 mg of Alizarin red S dye (sodium alizarinsulphonate) per litre of seawater (0.5 ppm). Alizarin red S is a calcium-specific dye (Newell 1982) and will produce a growth check or disturbance mark in bivalves (Hidu and Hanks 1968; Hurley et al. 1987).

Preliminary laboratory experiments showed that a dye concentration of 0.5 ppm was sufficient to produce an unstained growth check in postlarval giant scallop without severely hindering growth or causing excessive mortalities. The dye was incorporated into the animals' shell at higher concentration or during longer exposures; however, growth was arrested and high mortalities occurred.

The tank containing the collectors was aerated and kept at ambient water temperature (12°C). Scallop were batch-fed a 2:1 mixture of the unicellular algae *Chaetoceros calcitrans* and *Isochrysis galbana* for a final concentration of 85 cells·μL^{-1}·d^{-1}. After 3 d of immersion, bags were placed into 253-μm-mesh plankton nets for redeployment at the underwater site. Three bags were retrieved each week for 4 wk. Contents of the bags were washed, screened and initially preserved in seawater containing 5% buffered formalin, and then stored in ethanol.

Sorted scallop were prepared for microscopic examination and age determination by immersion in a dish containing 0.3% sodium hypochlorite (6% commercial bleach) for about 15 min. The valves were then teased apart using dissecting needles. After an additional 5 min of immersion, the soft tissue was easily removed from the valves. To validate the ageing technique, the number of ridges formed after the dye/disturbance mark (Fig. 1A) was counted for 20 scallops from each of the four grow-out periods. Left valves of the spat were placed, upright, in depression slides and the number of concentric ridges was counted using a compound microscope at 100 or 400× magnification.

Precision of repeated measurements of individual scallop (measurement error) and reproducibility of ageing between readers (counting bias) were determined by two readers who each aged 20 scallop. Scallop were independently aged five times by both readers for a total of 100 age determinations each (Table 1). Average percent error (APE) was calculated according to Beamish and Fournier (1981) and index of precision (D) and coefficient of variation (CV) were also calculated (Chang 1982).

Growth

Measurements of shell height (measured from the dorsal hinge line, i.e. the commissure and not the umbo, to the ventral edge) (Fig. 1B) and ridge width were made by placing a depression slide containing the left valves under a compound microscope with a video camera attachment. The image was projected onto a video screen which was integrated with a personal computer. Shell height and growth measurements were obtained using the Optimas image analysis software system. To facilitate measuring the ridges, several manipulations of the image were used to enhance image clarity, such as increased contrast and image "posterization." Video images are composed of an array of gray shades, and the "posterizing" feature reassigned the gray scale of a picture by reducing the image to the two extremes, i.e. black and white. Growth ridges could then be easily identified and measured on the "posterized" image compared with the unenhanced one (Fig. 1C and 1D).

Shell Morphology

A sample of 50 scallop was randomly selected from the four sampling periods and valves were cleaned and separated as described previously. Valve age since settlement was determined and valves were measured for larval (prodissoconch) shell height (from umbo to ventral edge of larval shell) (Fig. 1B), postlarval shell height, shell length (maximum anterio–posterior axis, parallel to the hinge line), and shell area, based on a planar view. All measurements were obtained using

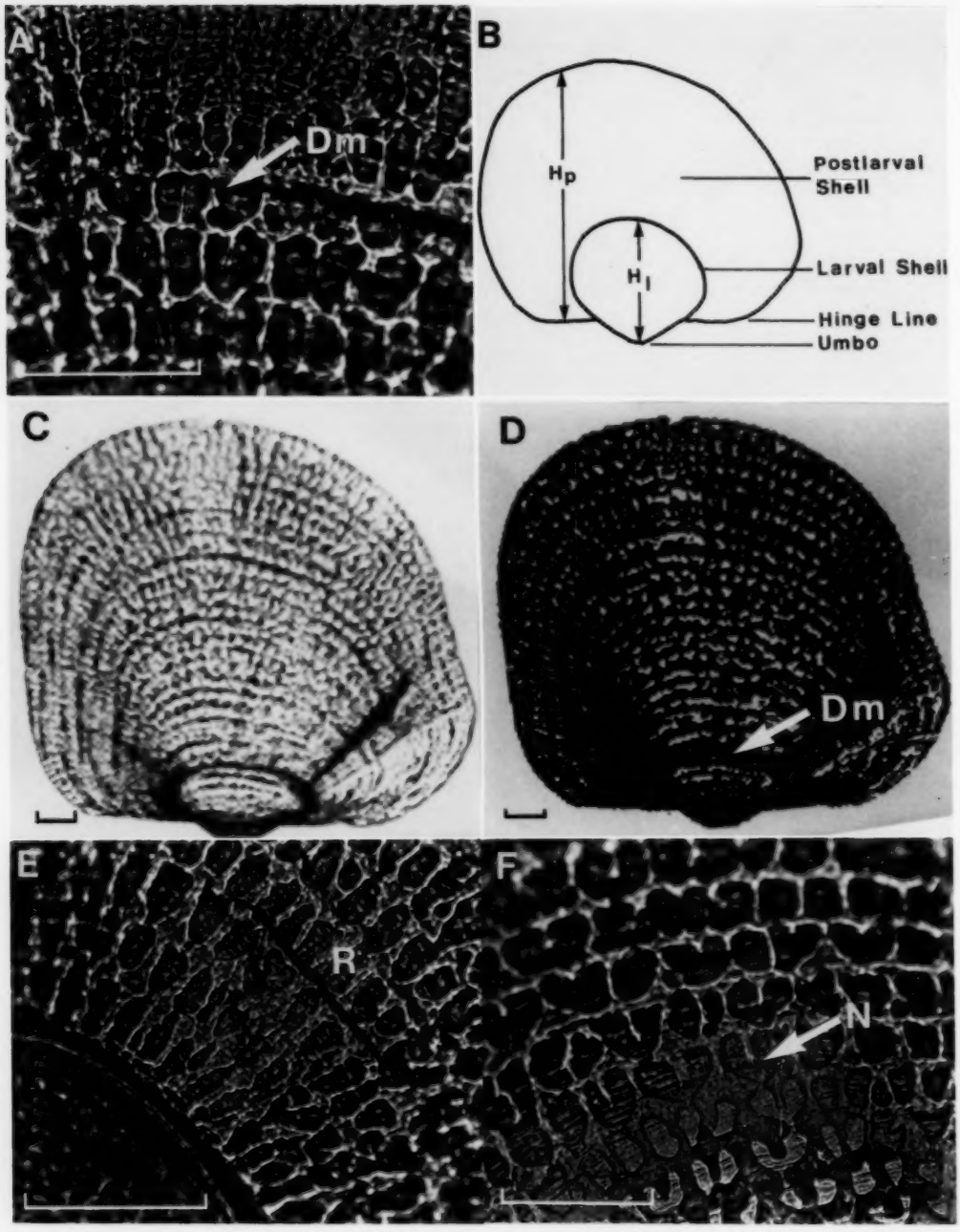

FIG. 1. Left valve of a postlarval giant scallop (scale bar = 100 μm). (A) Photomicrograph illustrating the disturbance mark (Dm); (B) schematic of a postlarva illustrating the larval shell, postlarval shell, hinge line, umbo, and the various shell height measurements; (H_l = larval shell height and H_p = postlarval shell height); (C) unmodified image of the left valve; (D) "posterized" image of the left valve of a 1-mm postlarval scallop; the disturbance mark is indicated by the arrow and the animal has 20 ridges after the disturbance mark; (E) photomicrograph of the concentric ridges (R); (F) photomicrograph illustrating the shapes of the individually raised nodules (N) on the surface of the left valve.

TABLE 1. Estimated total ages (d), average percent error (APE), coefficient of variation (CV), index of precision (D), and percent agreement in age determination for two readers of postlarval giant scallop shells.

	Reader 1								Reader 2							
No.	1	2	3	4	5	APE	CV	D	1	2	3	4	5	APE	CV	D
1	10	10	10	10	10	0.0	0.0	0.0	10	10	10	10	10	0.0	0.0	0.0
2	5	5	5	5	5	0.0	0.0	0.0	6	6	5	5	5	0.089	0.101	0.045
3	13	12	12	12	12	0.026	0.037	0.016	12	12	11	11	13	0.054	0.071	0.032
4	14	13	14	14	14	0.023	0.032	0.014	15	13	14	13	14	0.046	0.061	0.027
5	13	13	13	13	13	0.0	0.0	0.0	12	13	13	13	13	0.025	0.035	0.016
6	21	21	21	20	21	0.015	0.022	0.010	20	20	21	21	21	0.023	0.027	0.012
7	18	18	18	18	18	0.0	0.0	0.0	19	18	18	18	18	0.018	0.025	0.011
8	22	22	22	22	22	0.0	0.0	0.0	22	22	22	22	22	0.0	0.0	0.0
9	20	19	20	20	20	0.016	0.023	0.010	20	20	20	20	20	0.0	0.0	0.0
10	18	18	18	18	18	0.0	0.0	0.0	18	18	18	18	18	0.0	0.0	0.0
11	31	31	31	31	31	0.0	0.0	0.0	31	31	31	31	31	0.0	0.0	0.0
12	30	30	29	29	30	0.016	0.018	0.008	31	28	30	30	29	0.030	0.038	0.017
13	29	30	31	30	30	0.013	0.024	0.011	27	28	29	28	31	0.039	0.053	0.024
14	30	31	30	30	30	0.011	0.015	0.007	29	29	30	31	30	0.021	0.028	0.013
15	19	19	19	19	19	0.0	0.0	0.0	20	19	19	19	20	0.025	0.028	0.013
16	32	32	32	32	32	0.0	0.0	0.0	32	32	32	32	32	0.0	0.0	0.0
17	29	28	28	29	28	0.017	0.019	0.009	28	29	30	30	30	0.024	0.030	0.014
18	31	32	32	32	32	0.010	0.014	0.006	30	31	31	31	31	0.010	0.014	0.006
19	31	32	31	31	31	0.010	0.014	0.006	31	31	31	31	31	0.0	0.0	0.0
20	30	31	31	30	31	0.016	0.018	0.018	30	31	31	31	30	0.016	0.018	0.008
Mean						0.009	0.012	0.005						0.021	0.026	0.012

Percent agreement in age within ±0 = 58%
Percent agreement in age within ±1 = 92%
Percent agreement in age within ±2 = 100%

the image analysis system at a magnification of 40 or 100×. Calculation of shell area was facilitated by using an auto trace feaure of the software to measure outline of the valves.

Results

The left (upper) valve of newly settled postlarval giant scallop was translucent and the structure of its convex surface was composed of concentric ridges (Fig. 1E). Each ridge was formed by a series of adjacent, irregularly shaped nodules (Fig. 1F). In larger scallops, >1.5 to 2.0 mm, the shell became pigmented and ribbed and the nodules became elongated and formed continuous fine radial lines; as a result, the ridges were no longer discernable. The right valve of postlarval scallop was smaller and flatter than the left. It was translucent, smooth, and had an irregular prismatic structure that was not suitable for ageing.

The use of growth ridges for ageing giant scallop appears to be valid. The mean number of growth ridges agreed closely with age (days at large) for scallop from each of the four grow-out periods, indicating a low bias (high accuracy) for this method of age determination (Table 2). Other statistical methods also supported this assertion. The median and modal age were the same as actual age. A regression of estimated age (A_e) with actual age (A_a) was highly correlated and significant and was described by the equation

$$(1)\quad A_e = 0.996 \cdot A_a - 0.046$$

$$(r^2 = 0.997,\ n = 80,\ P < 0.001).$$

The mean number of ridges produced per day was not significantly different from 1.0 (t-test, $t = -2.05$, df = 79, $P > 0.05$).

The repeatability of age determinations was very consistent. The three indices of measurement error (average percent error, CV, and D) were all low, indicating a high degree of precision (Table 1). The CV for each reader was 1.2 and 2.6%, respectively, and 92% of all age determinations were within 1 d of each other, suggesting good agreement and low counting bias between readers (Table 1).

Mean daily growth rate of scallop, during the period of this study (September 29 to October 26, 1989), increased from 32

TABLE 2. Actual age (d), estimated mean, median, and modal age, range, standard deviation (SD), and coefficient of variation (CV expressed as %) of postlarval giant scallop held for four grow-out periods in Passamaquoddy Bay.

Sampling period	n	Actual age	Estimated mean age	Median	Mode	Range	SD	CV (%)
I	20	7	6.90	7	7	6–8	0.39	5.7
II	20	14	13.90	14	14	13–14	0.31	2.2
III	20	20	19.95	20	20	19–21	0.39	2.0
IV	20	27	26.80	27	27	26–27	0.41	1.5
Mean per day			0.99	1	1	0.86–1.14	0.32	3.2

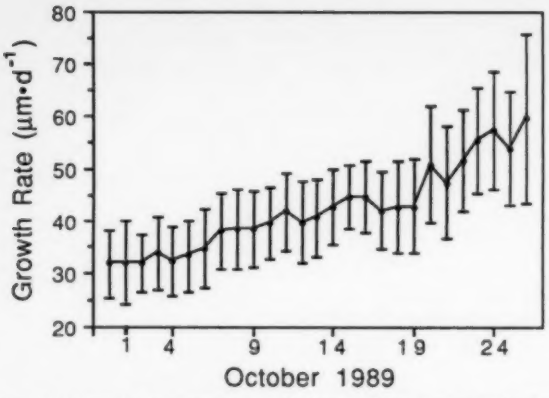

FIG. 2. Mean daily growth rate of giant scallop grown under field conditions from September 29 to October 26, 1989, at Tongue Shoal, Passamaquoddy Bay (vertical bars = ±SD).

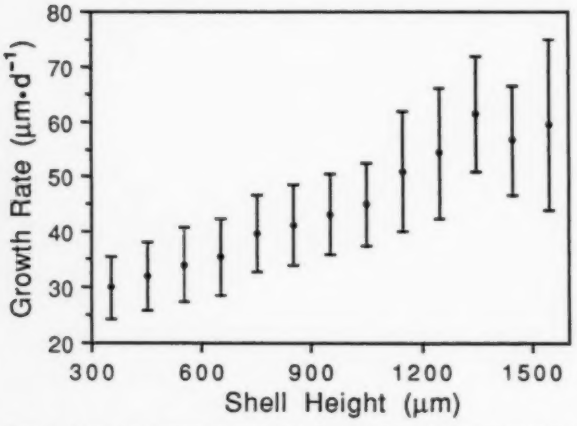

FIG. 3. Mean daily growth rate of giant scallop in relation to size (shell height) (vertical bars = ±SD).

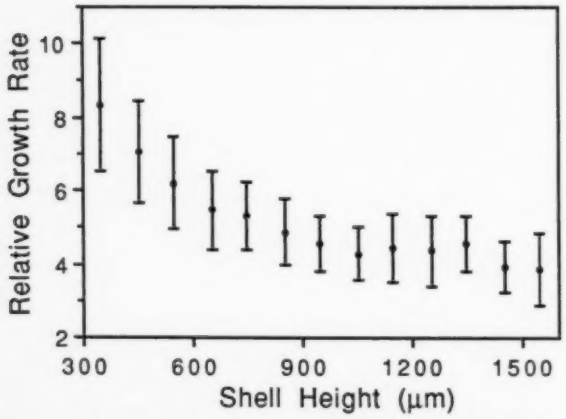

FIG. 4. Relative growth rate (percent growth per day) of postlarval giant scallop in relation to initial size (shell height) (vertical bars = ±SD).

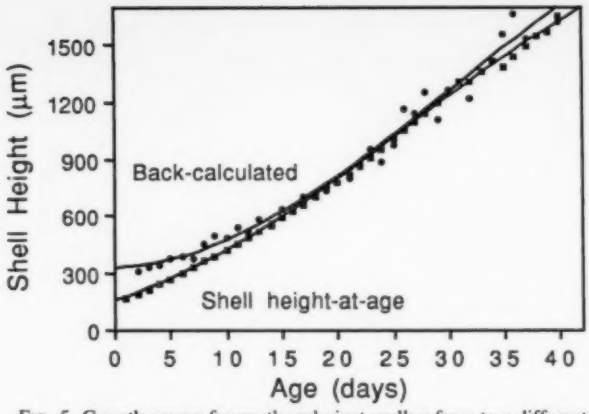

FIG. 5. Growth curves for postlarval giant scallop from two different methods of measurement: back-calculated shell height and shell height-at-age.

to 57 $\mu m \cdot d^{-1}$, respectively (Fig. 2). Differences in mean daily growth rate, while controlling for shell height, were significant (ANCOVA, $F = 3.365$, df = 26, $P < 0.001$). There was a positive relationship between daily growth rate and shell height, with growth rate increasing from 30 to 60 $\mu m \cdot d^{-1}$ for scallop ranging in size from 350 to 1600 μm, respectively (Fig. 3). The relative growth rate (percent growth from initial size per day) of scallop over the same size range exhibited an inverse asymptotic relationship to shell height (Fig. 4). Relative growth per day ranged from 8.3 to 3.8% for 350-1600-μm scallop, respectively.

Growth curves of postlarval scallop, based on two methods of estimating shell height, i.e. back-calculated shell height and actual shell height-at-age, were curvilinear (Fig. 5). The two relationships were similar except for a deviation in young postlarval scallop, where the actual mean shell height was lower than the back-calculated shell heights. As scallop grew, shell convexity increased. As a result, the apparent size of larval (prodissoconch II) shell (H_l) decreased with increasing postlarval (dissoconch) size (H_p) and was described by the regression equation

$$(2) \quad H_l = 282.8 - 0.144 \cdot H_p$$

$$(r^2 = 0.830,\ n = 189,\ P < 0.001).$$

This was due to the larval shell being rotated, from a horizontal, flat position to a vertical position (along the hinge axis) as the postlarval shell increased in height and convexity. Hence, the reference point from which shell height of the first several growth ridges were measured differed between the two methods, namely back-calculation and shell height-at-age. Shell heights obtained from the two methods were correlated ($r^2 = 0.979$, $n = 32$, $P < 0.001$), and the regression equation

$$(3) \quad H_a = 0.892 \cdot H_{bc} + 112.24$$

can be used to correct back-calculated estimates (H_{bc}) to actual shell heights-at-age (H_a).

To determine whether the growth of all individuals showed significant variation among individuals, regression analyses were conducted for all possible pairwise combinations among individual scallop for each grow-out period. The majority of the comparisons among the scallop were not significantly correlated (77%) or were not highly correlated (23%), indicating that all individuals in the experimental population did not show

TABLE 3. Summary of correlations of daily growth rates among postlarval giant scallop for each grow-out period.

Grow-out period	Number of scallop	Number of comparisons	Number of significant comparisons[a]	Range of r^2
Week 1	16	120	3	0.001–0.712
Week 2	18	153	8	0.001–0.590
Week 3	17	136	31	0.001–0.520
Week 4	16	120	80	0.001–0.819
Total		529	122	

[a]Significant at $P < 0.05$.

the same daily pattern of growth (Table 3). Even among the pairs of scallop that showed the highest correlations, a high degree of coherence was not observed (Fig. 6).

The relative change in shell proportions (shell height (H), shell length (L), and area) were described by the following power functions:

$$(4)\quad H = 0.035 \cdot L^{1.14} \qquad (r^2 = 0.989,\ n = 50,\ P < 0.001)$$

$$(5)\quad \text{Area} = 1.66 \cdot H^{1.91} \quad (r^2 = 0.998,\ n = 50,\ P < 0.001).$$

Both these relationships were allometric, i.e. slope was significantly different from 1.0 (H–L; t-test, $t = 8.2$, df = 48, $P < 0.05$) and from 2.0 (area–H; t-test, $t = 8.3$, df = 48, $P < 0.05$), respectively. Shell height – shell length allometry resulted in scallop having a greater height than length after a size of 1200–1400 μm.

Discussion

Concentric ridges in postlarval giant scallop have been previously reported (Merrill 1959, 1961; Waller 1972, 1991). Belding's (1931) study on the bay scallop (*Argopecten irradians*) suggested that these ridges may represent daily growth marks, but no studies on postlarval scallop have examined if ridges are indeed formed daily. Daily or near daily growth ridges have been reported for other life-history stages of several scallops, including larval *Placopecten magellanicus* (Hurley et al. 1987) and juvenile *Amusium balloti* (Joll 1988), *Argopecten irradians* (Wrenn 1972; Wheeler et al. 1975; Helm and Malouf 1983), *Chlamys opercularis* (Broom and Mason 1978), *Pecten maximus* (Antoine 1978), and *Pecten diegensis* (Clark 1968). In a number of these species, daily growth ridges persist only under "ideal conditions" (Clark 1974; Broom and Mason 1978; Palmer 1980). The periodicity and rate of ridge formation are reportedly influenced by size, age, temperature, and maturity (Antoine 1978; Gruffydd 1981; Joll 1988), thus limiting their use as an ageing tool. If growth ridges of postlarval scallop are to be used as a method of ageing, it will be limited to the preradial stage, i.e. <1.5–2.0 mm or 30–40 d old postsettlement, after which the concentric ridges become obscured by shell rib formation and pigmentation.

To validate the use of growth ridges as an ageing tool for postlarval giant scallop, it needs to be demonstrated that the ridges are formed on a periodic basis. The similarity between mean number of ridges and true age (i.e. low bias/high accuracy) and a low CV, 3.2% (i.e. high precision), indicate that this is a highly reliable method of age determination. The significant correlation between number of ridges and age (days at large) and the fact that the number of ridges produced per day was not significantly different from a value of 1.0 further support the validity of this technique.

The hypothesis that periodicity of ridge formation is daily was also supported by the above evidence which suggests that growth ridges were formed in synchrony with a daily cycle. Furthermore, according to the a priori criteria of Bourget et al. (1991) for evaluating such hypotheses, the hypothesis of daily growth ridges in postlarval giant scallop should be accepted. Firstly, Bourget et al. (1991) proposed that the variability about the mean should be explained. Few studies attempted to determine measurement error or error between observers (Peterson et al. 1983; Bourget and Brock 1990). In this study, the measurement error as determined by repeated measurements accounted for most of the variability in the data, i.e. the CV of mean age was similar to the CV of repeated measurements, 3.2 and 1.2–2.6%, respectively (see Tables 1 and 2). Furthermore, all estimated ages were within 2 d of the actual age, as were all age determinations between the two readers, i.e. the bias associated with the mean age was the same as the counting bias (between readers). Secondly, Bourget et al. (1991) stated that before accepting hypotheses on frequency of formation of growth marks, the effect of potential sources of variation should be evaluated. Studies on several species of bivalves have shown that age (Antoine 1978; Joll 1988), growth rate (Gruffydd 1981), habitat (Bourget et al. 1991), and season (Antoine 1978; Broom and Mason 1978) can influence growth mark formation. Growth ridges on postlarval giant scallop were only discernable up to ≈1.5–2.0 mm, but within this size range, growth ridge formation was independent of age (in days) and growth rate. Regional differences in frequency of growth mark formation have been reported for the cockle (*Cerastoderma edule*) (Bourget et al. 1991). Our study was conducted only in one location; thus, further studies need to be conducted on postlarval giant scallop in other regions to determine if daily growth ridges are a general phenomenon or only applicable to Passamaquoddy Bay.

If a hypothesis of periodic growth mark formation is rejected, one possible explanation may be "missing" marks due to environmental stress (Clark 1968). While methods have been developed to detect and correct for such anomalies (Clark 1968; Hughes and Clausen 1980), to compensate for such "missing" marks assumes that growth marks widths are correlated among specimens. Few studies have tested for coherence in growth mark widths among animals. Clark (1968) reported a correlation in increment width for the scallop *Pecten diegensis*, but Hughes and Clausen (1980) found no such relationship among the venerids *Chione fluctifraga* and *Protothaca staminea*. We found a poor fit in the growth ridge pattern among individual postlarvae. Hughes and Clausen (1980) postulated that a lack of correlation among animals from a similar environment may be due to size or age differences, microhabitat differences, or genetic factors.

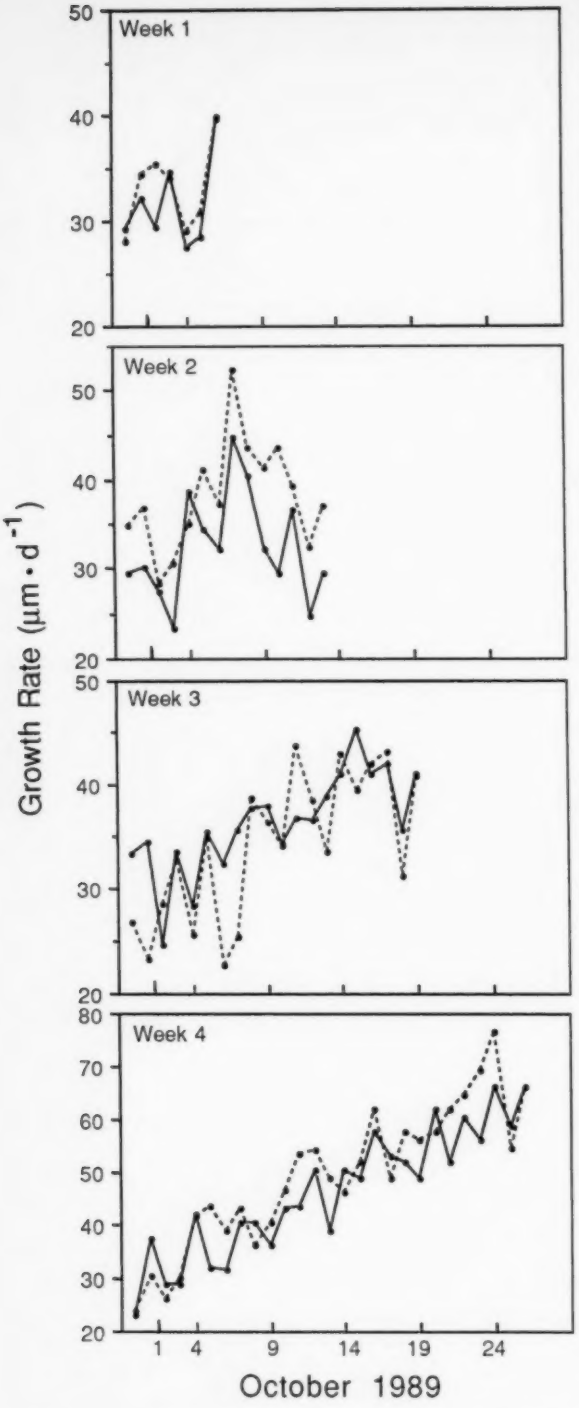

FIG. 6. Individual growth curves for two postlarval scallop from each grow-out period. Each pair of scallop represents the highest correlated pair for each grow-out period.

In our study, growth rate was related to both scallop size and age; however, comparisons of individual ridge widths were carried out on animals of a similar size and age for each grow-out period separately. Thus the lack of a pattern in ridge widths appears to be due to other factors. Postlarval scallop held at the Tongue Shoal aquaculture site were grown in artificial collectors. Animals within a collector were never more than 75 cm apart from each other, and scallop grown in different collectors, but from the same sampling period, were never more than 2 m apart. Within a collector, it is possible that water flow may be restricted and hence the encounter rate of food particles would be lower than that experienced at the periphery, but any changes would likely be relative (i.e. a pulse of food would generate an increase in growth both in the periphery and the center of the bag but not to the same degree). If microhabitat differences were responsible for the observed variation in individual growth rates, then the variability in the environment must have occurred on a fine scale, <2 m. To test if microhabitat differences are responsible, a study employing a fine-scale, high resolution sampling regime would be necessary. Clark (1974) suggested that the degree of response by individuals in increment widths may be due to genetic factors. This explanation could also be suggested for postlarval giant scallop, and to evaluate this hypothesis, a controlled laboratory experiment would be required (Hughes and Clausen 1980). Unfortunately, it was not possible to differentiate between microhabitat differences or genetic factors in explaining the lack of coherence in individual growth patterns among postlarval scallop from our data.

Our estimates of age determination for postlarval scallop were both accurate and precise and differed from studies on short-term internal growth increments in bivalve shells and larval fish otoliths. Age determinations for bivalve and larval fish internal increments are generally biased and have a precision of about 10–15% (Bourget et al. 1991; Campana and Moksness 1991). Several factors may explain the discrepancy between our study, using external ridges, and those using internal increments. Shell preparation for examining ridges was minimal and nondestructive compared with the preparation required for internal increments (see Rhoads and Lutz 1980). Sample preparation can introduce variability in age estimates for both bivalves (Farrow 1971; Hughes 1979) and larval fish (Campana and Moksness 1991). Increment number and widths in sectioned bivalve shells are not necessarily consistent throughout (Clark 1974; Hughes 1979) whereas in our study, ridge number and widths were radially consistent in most scallop. The width of external ridges were typically larger than increment widths in bivalves and larval fish. Because of their small size, increment clarity, resolution of narrow increments, and different orders of increments (e.g. "subdaily") can all complicate the interpretation of internal increments (Bourget et al. 1991; Campana and Moksness 1991).

Postlarval giant scallop had a mean daily growth rate which ranged from 32 to 57 $\mu m \cdot d^{-1}$ over the duration of this study. Merrill and Posgay (1967) and Merrill and Edwards (1976) found comparable growth rates of 27.4 and 15–73.3 $\mu m \cdot d^{-1}$, respectively, for young giant scallop up to 6 mo old from Nantucket Shoals. Larsen and Lee (1978) described a growth rate of only 4 $\mu m \cdot d^{-1}$ in 1-mm giant scallop found on Georges Bank in the winter. In a laboratory growth study, Sastry (1965) found similar rates of growth of 31–100 $\mu m \cdot d^{-1}$ for postlarval bay scallop (*Aequipecten irradians concentricus*). Growth rates for other species of postlarval scallops are varied and range from 14 $\mu m \cdot d^{-1}$ for *Chlamys (Chlamys) asperrimus* (Rose and Dix 1984) to 48–66 $\mu m \cdot d^{-1}$ for *Patinopecten yessoensis* (Maru

1985), 50 $\mu m \cdot d^{-1}$ for *Argopecten gibbus* (Allen 1979), 146–181 $\mu m \cdot d^{-1}$ for *Pecten novaezelandiae* (Bull 1976), and 290 and 340 $\mu m \cdot d^{-1}$ for *Pecten vogdesi* and *Argopecten circularis*, respectively (Ruiz-Verdugo and Cáceres-Martinez 1991).

Our growth rate estimates for postlarval giant scallop were consistent with the growth rates for giant scallop of all ages (e.g. Chandler et al. 1989). That is, our estimated postlarval growth rates were greater than those reported for larval giant scallop, 6.4 and 2.5 $\mu m \cdot d^{-1}$ by Culliney (1974) and Hurley et al. (1987), respectively, and were lower than those reported for juveniles, 120 and 90 $\mu m \cdot d^{-1}$ (Dadswell and Parsons 1991; Parsons and Dadswell 1992), and adults, <81 $\mu m \cdot d^{-1}$ (Chandler et al. 1989).

Our results indicate that the ridges on the surface of postlarval giant scallop from Passamaquoddy Bay are produced daily and this has several practical applications. By knowing the collection date of postlarval scallop, the number of daily ridges can be used to establish their settlement date and hence recruitment pattern to the benthos. Intra- and interannual daily growth rates can be obtained and their variability related to environmental (e.g. food and temperature) and biotic (e.g. survival and abundance) variables. These applications should enhance our understanding of the ecology of this hitherto little known life-history stage of the giant scallop.

Acknowledgements

We thank and appreciate those who assisted, especially F. Wilhelm and L. Colburn for field and laboratory work, C. Couturier for providing the cultured algae, F. Cunningham for preparing the figures and photographs, Dr. J. D. Neilson for use and instruction on the image analysis system, and Drs. J. D. Neilson and D. J. Wildish and two anonymous reviewers for commenting on the manuscript. The Department of Fisheries and Oceans provided office and laboratory space. Financial support was provided in part by an NSERC operating grant to J. C. Roff.

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Prey Retention and Sieve Adjustment in Filter-Feeding Bream (*Abramis brama*) (Cyprinidae)

Wim Hoogenboezem

Limnological Institute Vijverhof Laboratory, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands
and
Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Marijkeweg 40, 6709 PG Wageningen, The Netherlands[1]

Eddy H. R. R. Lammens[2] and Peter J. MacGillavry

Limnological Institute Vijverhof Laboratory, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands

and Ferdinand A. Sibbing

Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

Hoogenboezem, W., E. H. R. R. Lammens, P. J. MacGillavry, and F. A. Sibbing. 1993. Prey retention and sieve adjustment in filter-feeding bream (*Abramis brama*) (Cyprinidae). Can. J. Fish. Aquat. Sci. 50: 465–471.

The hypothesis that the variability in prey-size selection of filter-feeding bream (*Abramis brama*) is related to sieve adjustment was tested by feeding bream of approximately 20 and 30 cm standard length with daphnids. The size-selective feeding observed in experiments was compared with predicted selectivity curves derived from the channel width of branchial sieves, total filtered volume, and the retention probability of prey in the reduced or unreduced channels. The relationship between mesh size and retention probability of *Daphnia* of known length was determined empirically in sieving experiments using commercial sieves. The results with 20-cm fish demonstrated that the variability in prey selection of bream can be explained by the adjustment of its filter in two distinct mesh sizes. The relationship between filter adjustment and zooplankton availability was not always clear. The retention ability of large bream (>30 cm) was in close accordance with the model prediction for filter-feeding with reduced channels.

L'hypothèse selon laquelle la sélection de la taille des proies chez la brème (*Abramis brama*) est liée à la modification de l'orifice des fentes branchiales a été testée en donnant des daphnies à des brèmes dont la longueur standard variait entre 20 et 30 cm. La sélection des proies en fonction de leur taille, notée au cours des essais, a été comparée à la sélection prévue à partir de la largeur des fentes branchiales, du volume total filtré et de la probabilité de rétention des proies par des fentes branchiales réduites. La relation entre la taille des mailles et la probabilité de rétention de *Daphnia* de longueurs connues a été déterminé empiriquement à l'aide de tamis du commerce. Les essais portant sur les poissons de 20 cm de longueur ont montré que la variabilité de la sélection des proies chez la brème pouvait s'expliquer par la modification du filtre de ses fentes branchiales selon deux tailles distinctes. La relation entre la modification des fentes et la disponibilité du plancton n'était pas toujours nette. La capacité de rétention des grosses brèmes (plus de 30 cm) concordait de près avec les prévisions du modèle pour l'alimentation par filtration avec des fentes réduites.

Received August 6, 1991
Accepted September 22, 1992
(JB170)

Reçu le 6 août 1991
Accepté le 22 septembre 1992

Microcrustaceans form an important food resource for bream (*Abramis brama*) (Cyprinidae) in eutrophic lakes. At sufficiently high zooplankton densities, all size classes of bream feed on zooplankton, although retention ability differs between length classes of bream (Lammens 1985; Lammens et al. 1987). Filter-feeding bream sieve randomly engulfed water, the size selectivity being determined mainly by the mesh size of the branchial sieve (Lammens and Hoogenboezem 1991). Thus, adjustment of the sieve in bream can only be tested in filter-feeding specimens. In small bream or at low prey density, selectivity is influenced by a visually oriented particulate feeding mode (Jansen 1976; Lammens 1985; Sibbing 1991; Hoogenboezem et al. 1992). Some prey species, such as copepods or *Chaoborus* larvae, influence selectivity by a strong evasive behaviour, while cladocerans are less effective at escape from approaching predators (Drenner et al. 1978).

In short-rakered fish species such as bream the actual retention of particles was assumed to occur on the narrow serrated slit formed by the interdigitating gill rakers (Zander 1906; Lammens 1984; Sibbing 1991). However, no experimental evidence for this assumption was given. X-ray studies on the gill-arch movements of filter-feeding bream showed that the slits between successive arches were too wide according to the previous model for effective retention (Hoogenboezem et al. 1990). In addition, the slits between successive gill arches differed considerably, producing a heterogeneous filter system, indicating that retention according to this simple two-dimensional interarch slit model is unlikely (Hoogenboezem et al. 1990).

[1]Please send requests for reprints to this address.
[2]Present address: RIZA, Postbus 17, 8200 AA Lelystad, The Netherlands.

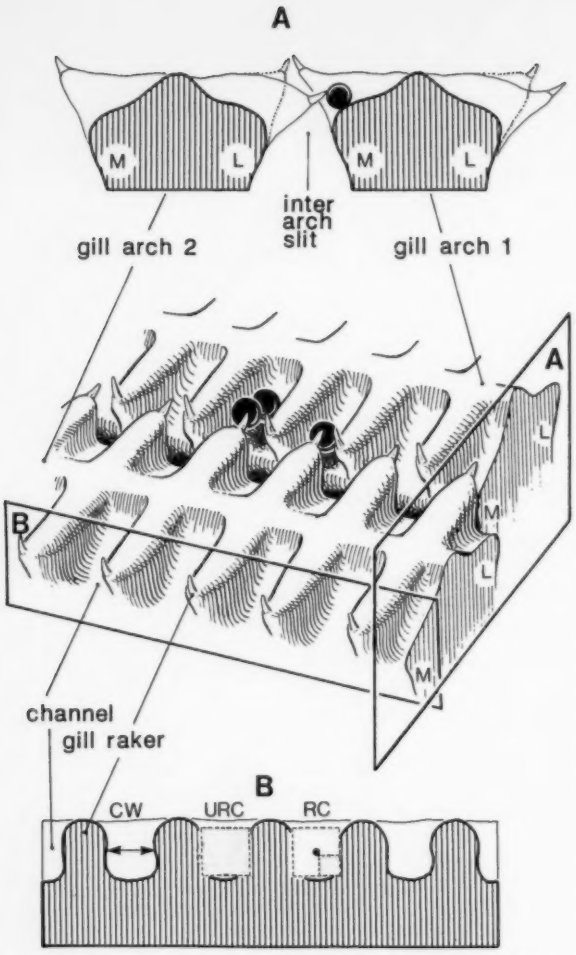

FIG. 1. Diagram of a part of two gill arches of the branchial sieve of bream. Note the channels and the site where small (0.5–1.0 mm) particles (black spheres) are retained. Plane A: cross section through two adjacent gill arches, indicating the lateral movable raker in the rest position (dotted line) and the raker inserted into the adjacent channel (solid line). Plane B: cross section through the channels; channel width (CW) was measured as indicated (arrow). URC = mesh size of the unreduced channel compared with the mesh of the commercial sieve; RC = reduced mesh size of the channel compared with a smaller mesh of the commercial sieve; the inserted raker tip is indicated as a black dot.

A detailed reconstruction of the branchial sieve morphology showed small channels on the upper surface of each branchial arch between its gill rakers. Each gill raker is composed of a cushionlike transverse ridge and a distal bony part. The spaces between two adjacent ridges form narrow channels. These channels are the sites of particle retention and form the mesh of the branchial sieve (Fig. 1). The presence of food particles in these channels supports this view. The path of marked particles was observed by X-ray cinematography which confirmed this mechanism of particle retention (Hoogenboezem et al. 1991).

The bony rakers on the lateral side of each gill arch possess a long spine and have a spindle-shaped base. Tiny striated muscles attach to these lateral rakers and rotate the raker outward

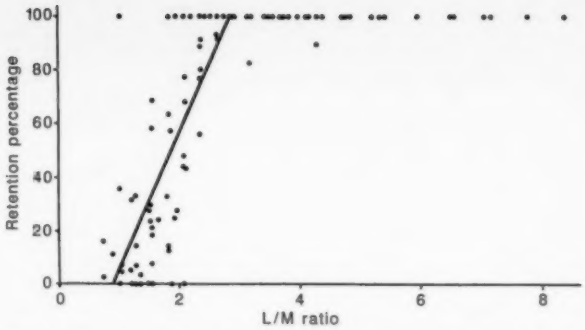

FIG. 2. Experimentally determined retention percentage (P_{cs}) of *Daphnia hyalina*, *D. cucculata*, and *Ceriodaphnia pulchella*, plotted as a function of the ratio of length to mesh size (L/M). The regression line was $P_{cs} = 0.57 \cdot (L/M) - 0.55$ ($n = 89$, $R^2 = 0.70$).

(Hoogenboezem et al. 1991). The medial rakers of each gill arch are not equipped with muscles and cannot rotate outward. Bream is assumed to reduce the mesh size of the channels by insertion of a lateral raker into a medial channel of the opposite gill arch (Hoogenboezem et al. 1991). No direct observation of channel reduction has been obtained to date, but previous feeding experiments showed that filter-feeding bream of intermediate size (mean length 20.2 cm) retained mainly large (>1 mm) cladocerans when their density was high, while smaller cladocerans (<1 mm) were not retained. A wider range (>0.5 mm) of prey was retained if the density of large daphnids was low (Hoogenboezem et al. 1991). These results were considered evidence for branchial sieve adjustment in filter-feeding bream.

Prey density, size composition of prey, size of fish, and light intensity determine whether the feeding mode is particulate or filter feeding (Lammens and Hoogenboezem 1991; Hoogenboezem et al. 1992). Within the filter-feeding mode, bream has the option to reduce the channels and select a wider range of prey than is possible in the unreduced mode. We think that the availability of both small and large prey will determine whether channels will be reduced or not. However, at this time, it is not possible to calculate all the energy constraints of food handling with reduced and unreduced channels, and therefore, it is not possible to predict sieve adjustment in a specified trial a priori.

In the present study, we will test the reducible channel model by comparing observed retention curves of *filter-feeding* bream with predicted curves based on unreduced (URC) and reduced channels (RC). If the observed retention curves are equal to or between those predicted by URC or RC, the above model is supported by the experimental data. For the feeding trials, we used relatively large fish and a nonevasive prey type (*Daphnia* spp.) at relatively high density to preclude particulate feeding. The predicted retention curves were based on the mesh size of the channels, the volume of water filtered, and the retention probability of prey items in the channels, based on their retention in commercial metal sieves.

Material and Methods

Sieving Experiments and Prediction of Retention Probability

The retention probability of cladocerans in relation to mesh size was determined with commercial sieves (10 cm, diameter, inflexible metal). One litre of live zooplankton was poured over a sieve with known mesh size (0.210, 0.285, 0.430, or

0.750 mm). The sieve was washed with 1 L of plankton-free water and the filtrate was collected on a 0.060-mm sieve. Length of zooplankton was determined from both fractions. Cladoceran lengths were measured to the nearest 0.02 mm using an ocular micrometer and were arranged in length classes of 0.11 mm.

The retention probability of each *Daphnia* length class in a commercial sieve (P_{cs}) is calculated as the ratio between the number of daphnids retained to total number of daphnids. If *Daphnia* is isometric in relation to length, P_{cs} will be determined by the relationship between *Daphnia* size and mesh size. The ratio of *Daphnia* length to mesh size (*L*/*M* ratio) was therefore assumed to determine the P_{cs}. The results of the sieve experiments were used to determine the *L*/*M* ratio.

The mesh used in the sieving experiments was square, while the channels in bream are rounded in section (Fig. 1). To determine the theoretical retention probability of a given prey in the branchial sieve, the URC width was used as *M* in the *L*/*M* ratio (Fig. 1). The retention probability in RC was based on calculation of restricted cross-sectional area resulting from insertion of a gill-raker tip (Fig. 1) into the centre of the channel.

Channel Diameter Measurements

Channel diameters were measured in a series of 30 bream, ranging from 3 to 43 cm standard length (SL), caught in Lake Tjeukemeer, a shallow, eutrophic lake in the north of The Netherlands. The captured bream were immediately preserved in formalin (10%) for 14 d prior to measurement. Branchial sieves were dissected from the fish and measured. van den Berg et al. (1992) have shown that shrinkage due to fixation is only approximately 4%. In each gill arch, five separate channels were measured in the central area of the arch (Fig. 1) to calculate the mean channel width, and one fish per size class was measured. In one fish (31 cm SL), which was used in some of the feeding experiments, all channels in the left half of the sieve were measured (van den Berg et al. 1992).

Feeding Experiments: Observed Retention Ability (P_{obs})

Bream were caught in Lake Tjeukemeer and acclimated in aquaria at 18°C for at least 6 wk before the feeding experiments started. During this period, bream of various lengths (18.0, 20.0, 21.6, 21.8, 22.1, 23.5, 29.5, 30.0, 34.3, and 34.5 cm SL) were fed live zooplankton, which is the main food of all length classes of bream in May and June and usually in September (Lammens et al. 1985). The experiments were conducted in aquaria (180 × 60 × 60 cm for bream 29.5–34.5 cm or 90 × 60 × 60 cm for bream <30 cm), each containing one bream. The fish were starved at least 24 h before the experiments, and in an earlier study, we checked satiation effects in long-lasting feeding experiments with a continuous high density of daphnids. Only after 8 h of feeding did intensity of a 24-cm bream decrease (Hoogenboezem et al. 1992). All the experiments were carried out in fully illuminated aquaria (two 36-W fluorescent lamps just above the water surface).

Zooplankton was collected with a hoopnet (125- or 250-μm mesh size) from the lake and transported in buckets to the laboratory. From the freshly collected zooplankton, the injured daphnids were removed in about 30 min. These daphnids usually had air between their valves and did not swim normally but floated on the surface. Only undamaged zooplankton was added to the experimental tank. Prior to the feeding experiment, zooplankton density and size distribution were determined by taking 12 or 20 (in the larger tank) water samples of approximately 200 mL with a perspex tube (5 cm diameter, 40 cm length) with a stopper. The samples were filtered through a 0.060-mm sieve and the zooplankton was preserved in 4% formalin. The volume of sampled water was measured in a calibrated cylinder to the nearest 5 mL. The length of individual zooplankton excluding caudal spine and helmet was measured with an ocular micrometer to the nearest 0.02 mm. The zooplankters were grouped by species in successive length classes of 0.11 mm. The sampling procedure was repeated after a foraging time of 2–4 h.

The retention abilities (P_{obs}) of the experimental fish were calculated per zooplankton size class as the ratio between the number of zooplankters before and after the experiment:

$$(1)\quad P_{obs} = \frac{N_0 - N_t}{N_0}$$

where N_0 = number of zooplankton at the start of the trial and N_t = number at the end.

Estimation of Feeding Efficiency and Filtered Volume

The decrease of zooplankton in the experimental tank depends on the water volume, zooplankton density, buccal volume of the fish, snap frequency (Hoogenboezem et al. 1992), foraging time, and size-dependent retention probability of prey items in the branchial sieve. A filter-feeding bream takes a series of snaps, each snap encompassing a certain volume of water containing prey. During the experiment the number of prey decreases exponentially, depending on the above variables. The expected number of prey items remaining after the experiment can be calculated for each size class by

$$(2)\quad N_t = N_0 \cdot e^{-((BV/AQ)\cdot SF\cdot RF\cdot t)}$$

where N_0 = number of prey present in the tank at $t = 0$, N_t = number of prey in the tank after t seconds, BV = buccal volume (litres), AQ = aquarium volume (litres), SF = snap frequency (snaps per second), RF = retention probability of prey in the channel, and t = duration of the experiment (seconds).

Since exact data for SF over the total experimental period were not available, snap frequency was derived by measuring the decrease of the largest prey items which were 100% retained (RF = 1) in the branchial sieve (equation 3); the minimum prey size to achieve 100% retention was derived from the results of the sieving experiments and the channel width measurements by using the *L*/*M* ratio, the minimum size depending on the size of the fish (1 mm for 20 cm, 1.4 for 30 cm bream):

$$(3)\quad \frac{\ln (N_t/N_0)}{-(BV/AQ)\cdot t} = SF\ (\text{snaps}\cdot\text{s}^{-1}).$$

This mean calculated SF for larger prey (RF = 1) is applied in equation 2 to calculate the expected number of prey (per size class) left after the experiment. The retention probability in the branchial sieve for all size classes (all RF values) is calculated for both URC and RC for all trials separately.

Calculations were made to determine the expected retention ability (equation 1) for URC (P_{URC}) and RC (P_{RC}) using the expected numbers of prey at the end of the experiments. The mean observed retention curve, for each size class, of several experiments was plotted in one figure together with the predicted retention curves, which were calculated from the model.

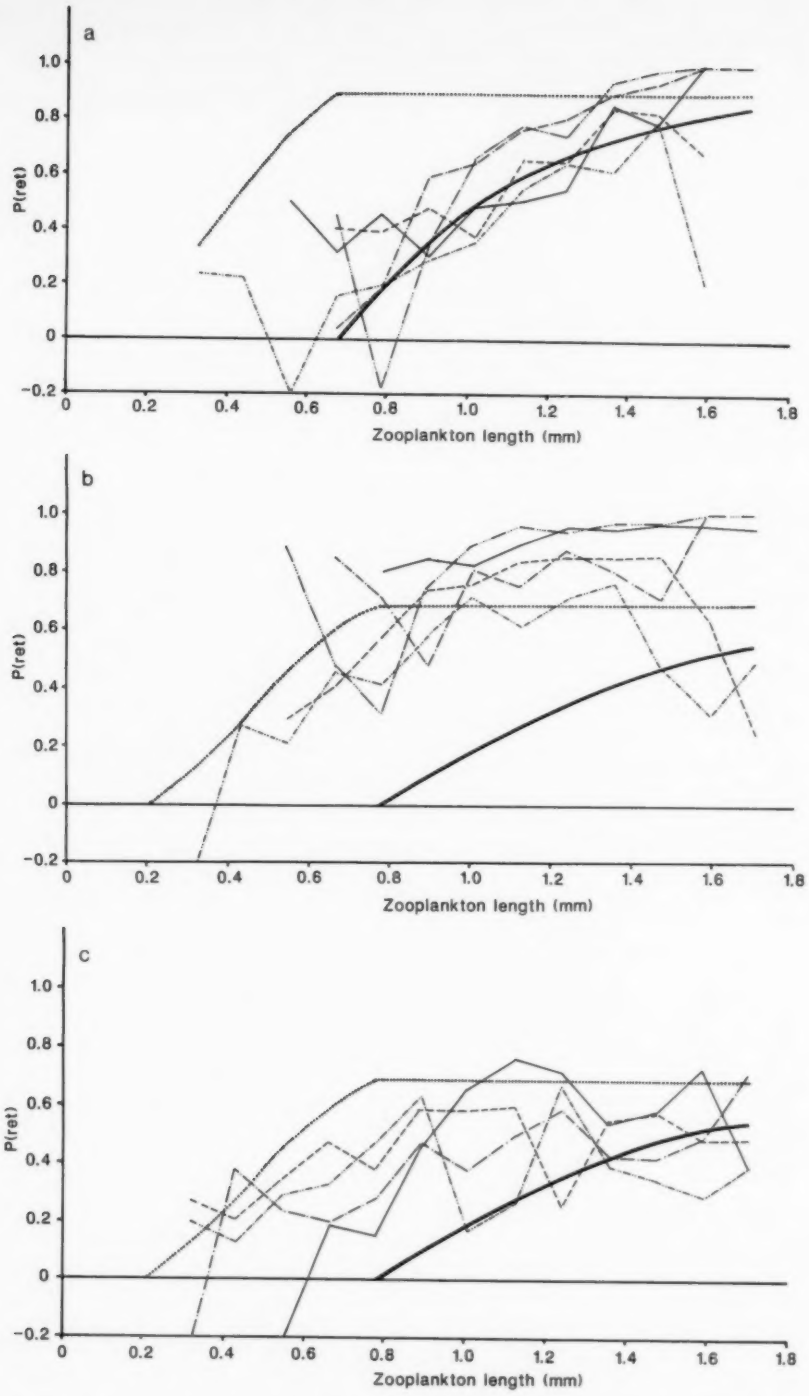

FIG. 3. Observed retention percentage (P_{obs}) of bream (18.0–23.5 cm SL) feeding on daphnids of different length classes, as a function of prey length. The dotted line represents the retention curve predicted from the model for reduced channels (P_{RC}) and the thick solid line represents the retention curve predicted for unreduced channels (P_{URC}). (a) Five trials corresponding to feeding with URC; (b) five trials corresponding to feeding with RC; (c) four trials representing a selectivity between URC and RC filtering.

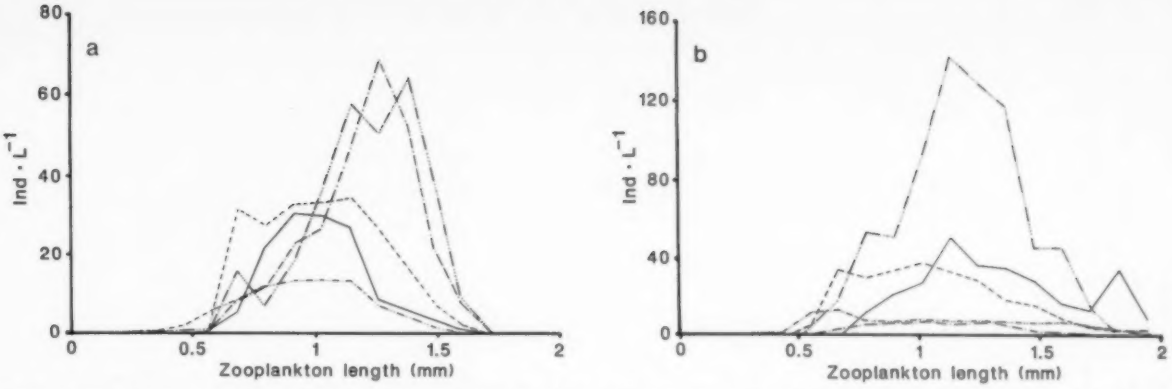

FIG. 4. Length frequency graph of cladocerans at the beginning of the feeding experiments with bream (18.0–23.5 cm SL). (a) Unreduced channel filter-feeding; (b) reduced channel filter-feeding (compare with Fig. 3a and 3b, respectively).

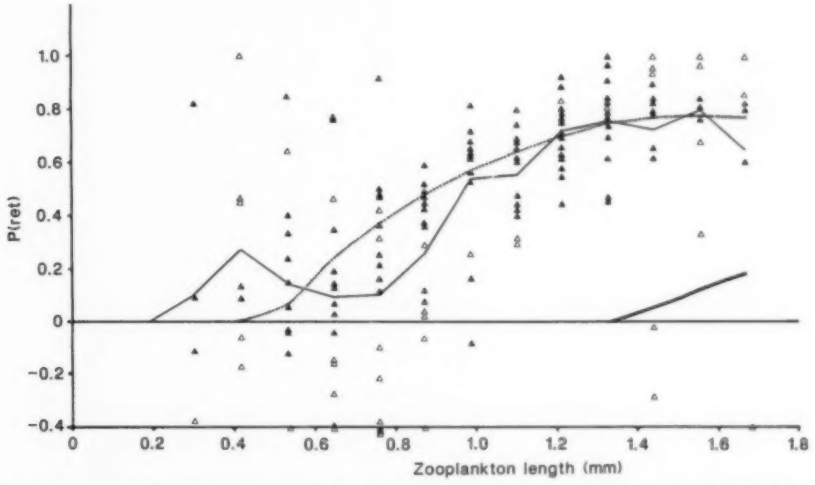

FIG. 5. Observed retention percentage (P_{obs}) in 16 feeding experiments with large bream (30.0–34.5 cm SL) feeding on cladocerans of different length classes, as a function of prey length. The dotted line represents the retention curve predicted from the model for reduced channels (P_{RC}), the thick solid line represents the retention curve predicted for unreduced channels (P_{URC}), and the thin solid line is the mean observed retention (P_{obs}). The triangles indicate all individual retentions per size class of all experiments. Retention percentages based on low prey density (≤ 5 ind·L^{-1}) are marked as open triangles. The occurrence of negative retention, especially for the relatively rare smallest and largest prey items, is due to measurement errors. Triangles plotted on the -0.4 axis were occasionally even lower than -0.4.

Results

Experiments with Commercial Sieves

The retention probability for *Daphnia* spp. having a length >2.5 times the mesh size (width of square mesh) of the sieve is 100% ($P_{cs} = 1$). Specimens of the same length as the mesh width ($L/M = 1$) were seldom retained ($P_{cs} < 0.15$). Between L/M ratios of 1.0 and 2.5 the probability of retention sharply increases. The measured retention ratio ascends more or less linearly to 100% (Fig. 2). This linear relationship was used to calculate the expected retention probability of prey items in the feeding experiments, using the length of the *Daphnia* and the channel widths (either URC or RC) as variables in the L/M ratio.

Channel Diameters

Based on the width of the interarch slits during feeding (Hoogenboezem et al. 1990), approximately 90% of the water was estimated to flow through the first two slits. The slit between arches 1 and 2 is approximately 31 mm long and 2.4 mm wide and represents approximately 68% of the slit surface at maximal abduction in a 31-cm bream. The slit between arches 2 and 3 is slightly shorter (approximately 30 mm) and distinctly less wide (0.86 mm) and is therefore estimated to conduct only 22% of the water (Hoogenboezem et al. 1990, 1991). The widths of the medial channels in slits 1 and 2 are similar (1.26 and 1.28 mm, respectively, in a 31-cm bream).

The single fish in which all channels in the left half of the sieve were measured showed some variation in channel widths.

Especially in the area where the branchial arches (epibranchial area) were connected to the skull, smaller channels were found (arch 1 medial channels 0.64 mm; SD 0.19, $n = 7$). The channels in the central area were larger and more regular in width (arch 1 medial channel 1.26 mm; SD 0.03, $n = 16$). A decrease in channel width, central area, from the first arch towards the fourth arch (medial channel 0.70 mm; SD 0.16, $n = 10$) was found (van den Berg et al. 1992).

The channel width (CW), measured in the central area of the gill arch, in bream is linearly related to fish size (Van den Berg et al. 1992) for the medial channels of arch 1:

$$(4)\quad CW = (0.046 \cdot SL) - 0.104 \qquad (SL \text{ in cm};\ n = 150,\ R^2 = 0.94).$$

In model calculations, the calculated CW of the medial channels (arch 1) was used, since approximately 90% of the water apparently flows through slits with channels of this diameter.

Feeding Experiments: Observed Retention Ability (P_{obs})

For 18.0- to 23.5-cm bream, predictions for both RC and URC were confirmed, but some results were also intermediate (Fig. 3). In five trials the pattern of retention by size class of cladocerans corresponded to the prediction for the URC model (Fig. 3a), and in five others it was consistent with the RC model (Fig. 3b). Four experiments, however, were intermediate (Fig. 3c), which may indicate a switch from URC feeding to foraging with RC. Occasionally a negative retention percentage was observed, especially in the less abundant size classes, which is considered to result from the low numbers (≤ 5 ind·L^{-1}) of these prey items present in the tank.

While it was only possible to predict the difference in prey retention of bream which filter fed with URC or RC, it was not possible to relate type of retention to food density or size. Therefore the experiments were grouped in two classes according to their similarity with the prediction of the URC or RC curve. A third group comprised the intermediate curves. The food availability in the experiments was constantly high (80–1600 ind·L^{-1}) to evoke filter feeding. Filter feeding with URC is expected to occur at relatively high density of larger prey (>1.0 mm). At low density of this prey size, filtering with RC is considered as most probable. Some of the results, however, were distinctly different. The group of URC trials included unexpectedly a trial with a low prey density (Fig. 3a, 4a, plot — ·· —), and one of the RC trials appeared to be carried out with a very high density (Fig. 3b, 4b, plot — ···· —). To induce exclusively RC filtering would require much lower densities, but in such circumstances, particulate feeding will occur, thus spoiling the filter-feeding experiments (Hoogenboezem et al. 1992).

For bream >30 cm the retention curve derived from the model for particles in the unreduced channels is very low (Fig. 5), the largest prey items in the experiments having a retention probability of approximately 0.2 in the URC model prediction. As expected, all experiments using large bream (30.0–34.5 cm) indicated reduced channel foraging (Fig. 5) at densities varying from 30 to 1390 ind·L^{-1}. The mean P_{obs}, based on all measured data, fitted the expected RC curve very well. Some individual trials deviated slightly from the RC model curve, but the observed retention probability was still considerably higher than that from the URC model curve. At low prey density counting errors may have a strong influence on the calculation of the retention percentage. Therefore, P_{ret} was plotted with a different symbol (open triangles) for zooplankton densities ≤ 5 ind·L^{-1} (Fig. 5).

Discussion

Mechanism of Particle Retention

The retention probability of particles in a sieve depends on at least three factors: (1) the mesh size and homogeneity of the sieve (Boyd 1976; Wright et al. 1983; Drenner et al. 1984; Gibson 1988), (2) the size and shape of the particles (Wright et al. 1983; Gibson 1988), and (3) the orientation of the particle when approaching the mesh of the sieve.

The homogeneity of the sieve expressed as a cumulative frequency of different interraker distances may serve as a retention probability curve (Boyd 1976). It assumes an equal flow of water through every part of the sieve, but this is not necessarily the case in bream. In the branchial sieve of bream the size distribution of the channels appeared to be related to the position on the arch. Only a few (six to eight per arch) distinctly smaller channels are located in the upper (epibranchial) part of each arch; they are partly or completely concealed by the large cushionlike palatal organ (cf. Sibbing and Uribe 1985), and it is very improbable that equal volumes of water will flow through these channels. The channels lying in the horizontal part of the branchial sieve show only minor variation in diameter. Therefore, a constant mesh size for each fish was used in the model calculations. Moreover, the mesh size was taken from an area where most of the water was filtered (i.e. medial channel arch 1).

The orientation of approaching particles cannot be quantified, since it is impossible to know the orientation of each particle. Due to this uncertainty, it is expected that a particle has a retention probability rather than a fixed value. Both Wright et al. (1983) and Gibson (1988) were unable to determine an appropriate measure of the prey items to estimate the retention probability reliably. In this study the empirically determined P_{cs} by means of the L/M ratio appeared to be useful. From the present results the channel width, or RC width, seems to be a suitable measure for the mesh size of the branchial sieve.

Predictive Value of the Channel Model

Intermediate-sized bream (18.0–23.5 cm) appeared to filter both in the RC and URC modes. In several feeding trials, bream showed URC feeding, and in others showed distinct RC feeding. In four experiments the position of the observed retention curve was between those of the URC and RC model curves, suggesting that these fish switched from one mode to the other during the experiment.

On the basis of the present data, we cannot explain how the choice of mode depends on food availability. To ensure filter feeding in this size class of bream, high zooplankton densities are required. Most probably, RC filtering becomes very important in nature at densities about one fifth those of the experimental conditions. Filtering with RC has the advantage of retaining more food particles, but it increases the resistance of the sieve, demanding more handling costs. As bream envelope the strained zooplankton in mucus (Hoogenboezem and van den Boogaart 1993), the handling time may be limited by mucus production. Therefore, in this size class of bream the net energy gained using either mode is probably similar at the offered concentrations of food, and therefore the fish use either mode.

Bream >30 cm have channels >1.2 mm, too large for URC feeding on the offered zooplankton, which was confirmed by

the feeding experiments where all fishes fed with reduced channels. Consequently, bream of 40 cm or larger are probably unable to feed on zooplankton efficiently. This may explain why in Lake Tjeukemeer, where the density of large daphnids (>1.4 mm) is usually low (Vijverberg and Richter 1982; Lammens et al. 1985; Vijverberg et al. 1990) and alternative benthic prey is scarce, bream rarely exceed 40 cm in length. In the IJsselmeer, bream attain a maximum size of approximately 60 cm because they have the opportunity to switch to chironomids and oligochaetes, which are more abundant there (Heermans 1978).

The retention probability of the branchial sieve of bream appeared to be reasonably predictable by the channel model. The present results confirm predictions from the channel model, but the mechanism that triggers the adjustment of the filter in foraging bream is not yet clear.

Acknowledgements

We thank Jan W. M. Osse (Wageningen), Koos Vijverberg (Nieuwersluis), Jos G. M. van den Boogaart (Wageningen), K. D. Hyatt (Nanaimo, B.C.), and an anonymous referee for their helpful suggestions to improve the manuscript. Jos G. M. van den Boogaart prepared the figures. The investigation was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for Scientific Research (NWO Project 811-437-241B).

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Importance of Mucus in Filter-Feeding of Bream (*Abramis brama*)

Wim Hoogenboezem

Limnological Institute Vijverhof Laboratory, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands
and
Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

and Jos G. M. van den Boogaart[1]

Agricultural University, Department of Experimental Animal Morphology and Cell Biology, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

Hoogenboezem, W., and J. G. M. van den Boogaart. 1993. Importance of mucus in filter-feeding of bream (*Abramis brama*). Can. J. Fish. Aquat. Sci. 50: 472–479.

Mucus boluses, found in the oropharyngeal cavity of a large number of freshly caught bream (*Abramis brama*), appear to play an important role in the aggregation and transport of food particles from the branchial sieve to the esophagus. Mucus boluses, some containing up to 900 zooplankters, were isolated from the oropharyngeal cavity of bream, and from these the filtering rate and retention ability of the filtering system were calculated. Using light microscopy and special staining methods, we identified different types of mucus cells in the oropharyngeal wall of bream that increase in number from anterior to posterior. Based on these data and on our knowledge of branchial sieving, we have hypothesized the role of mucus in the filter feeding mechanism of bream. Initially, small particles are retained mechanically in the branchial sieve, eventually becoming coated with mucus. They then act as "sticky" particles with respect to other mucus-coated particles. During recollection from the branchial sieve following closed protrusion of the mouth, the sticky particles adhere to one another forming a mucus bolus that is stored at the posterior end of the branchial chamber. After several of these cycles over a period of several minutes the bolus is swallowed.

Les bolus de mucus trouvés dans la cavité oropharyngienne d'un grand nombre de brèmes (*Abramis brama*) peu de temps après leur capture semblent jouer un rôle important dans l'agrégation et le transport des particules alimentaires entre le filtre branchial et l'oesophage. Des bolus de mucus, dont certains contenaient jusqu'à 900 organismes zooplanctoniques, ont été isolés de la cavité oropharyngienne de brèmes et ont servi à calculer le taux de filtration et la capacité de rétention de l'appareil filtreur. Nous avons pu identifier dans la paroi oropharyngienne, à l'aide du microscope optique et de techniques de coloration particulières, divers types de cellules mucoïdes dont le nombre augmentait dans l'axe antérieur–postérieur. À partir de ces données et de nos connaissances du filtrage branchial, nous avons formulé une hypothèse quant au rôle du mucus dans le mécanisme d'alimentation par filtrage des brèmes. Les petites particules sont tout d'abord retenues de façon mécanique par le filtre branchial, après quoi elle sont recouvertes de mucus. Ces particules peuvent donc maintenant s'agglutiner aux autres particules recouvertes de mucus. Pendant la récupération des particules du filtre branchial, suite à la fermeture vers l'avant de la bouche, les particules s'agglomèrent et forment le bolus qui se déplace vers le fond de la cavité branchiale. Ce cycle se répète plusieurs fois en une période de quelques minutes, après quoi le bolus est ingéré.

Received October 17, 1991
Accepted September 22, 1992
(JB272)

Reçu le 17 octobre 1991
Accepté le 22 septembre 1992

In order to understand the mechanisms for particle retention and transport in filter-feeding fishes, it is important to consider the role of oropharyngeal mucus. Drenner et al. (1982) reported the presence of mucus-bound zooplankton in the gizzard shad (*Dorosoma cepedianum*), but no details on numbers or frequency of these observations are available. This early observation also lacks information on its possible role. Several other authors have proposed mechanisms for particle retention by means of mucus (Greenwood 1953; Matthes 1963; Drenner et al. 1987; Northcott and Beveridge 1988; Beveridge et al. 1989). Sanderson et al. (1991) endoscopically observed small aggregations of food particles and mucus in Sacramento blackfish (*Orthodon microlepidotus*), but it is not clear whether these aggregations were a result of a retention function of the mucus or whether the mucus performed a role in transporting the retained particles, or both.

Sibbing and Uribe (1985) conducted a detailed study on the role and structure of oropharyncheal mucus in the common carp (*Cyprinus carpio*). They identified two types of mucus cells with distinct differences in size, shape, position, and contents in the oral epithelium. Both cell types secrete acid mucus. A small pyriform cell type contains sialomucines and is evenly distributed throughout the oropharyngeal cavity. According to Sibbing and Uribe (1985), the function of these cells is to reduce the boundary layer and water flow resistance and also to prevent damage to the epithelium. A large sacciform type of mucus cell contains sulphomucines and predominates in the posterior pharyngeal region. It has the same function as the former cell type and additionally serves in the transport and aggregation of food particles into a sulphomicine mucus bolus just before swallowing.

Bream (*Abramis brama*), a common cyprinid in Dutch eutrophic waters, forages effectively on benthic chironomids and zooplankton during its entire life span (Lammens 1985;

[1]Author to whom reprint requests should be addressed.

Lammens et al. 1987; Lammens and Hoogenboezem 1991). Relatively large specimens (up to 30 cm fork length (FL)) are able to retain even small cladocerans (>0.5 mm) in the channels of the branchial sieve. The branchial sieve of bream is formed by two series of gill rakers on the first four branchial arches. Each gill raker is composed of a cushionlike structure forming the transverse ridge and a distal bony part. The space between two adjacent transverse ridges forms a channel in which particle retention occurs. When enough large prey items are available, bream forage with unreduced channels (URC), but when only small prey are available, bream are able to adjust the mesh size of the filter by reducing the channel diameter (Hoogenboezem et al. 1990, 1991).

Retained food particles have to be transported from the branchial sieve to the esophagus. Because these particles may easily be lost through the large interarch slits (Hoogenboezem et al. 1990; Van den Berg et al. 1992), some mechanism must exist to prevent this. The presence of mucus boluses containing zooplankton in the oropharyngeal cavity of bream suggests that mucus may play an important role in the processing of retained zooplankton. In this study, we describe the types and distribution of mucus-producing cells in the branchial sieve of bream, the mechanisms that cause mucus release, and the role of mucus in the collection and transport of particles captured from the branchial sieve system.

Materials and Methods

Bream were collected by trawling (net mesh size 5.5 mm) from two different lakes in Friesland in the north of The Netherlands. Two or three trawls were conducted per sampling day during April–November 1988. During the same period, regular water samples were taken at the sites where the trawls had begun and/or ended using a 5-L Friedinger sampler (c.f. Lammens et al. 1985). The water samples were used to estimate the density and size distribution of the zooplankton. Bream were measured (FL) and assigned to rough length classes (FL < 15 cm, 15 < FL < 30 cm, and FL > 30 cm).

Collection of Mucus Boluses

Mucus boluses were either collected from the oropharyngeal cavity of freshly killed bream after dissection or obtained by washing them out of the mouth cavity of live fish. This was done using an artificial backflow of water generated by a squeeze bottle through a hollow perspex wedge that had the shape of the opercular slit (Fig. 1). The volume of the boluses was measured in water to the nearest 0.1 mL using a narrow calibrated glass cylinder. Afterwards the boluses were either fixed in 4% formaldehyde solution or stored frozen at −20°C. Average volume (± SD) of the mucus boluses was calculated for each length class of fish that was present in each catch. This provided data on average FL versus average bolus volume throughout the season. On one sampling day (in the first week of June 1988), measurements of each individual fish and its bolus were kept separate.

Calculation of Filtering Rate and Retention Ability

After partially dissolving the mucus by gently stirring in 4% potassium hydroxide solution, the zooplankton in the mucus boluses, as well as in the water samples, were identified, measured, and counted. The filtering rate (i.e. the filtered volume of water required to retain the zooplankton) and the retention ability of the fish could be calculated because the distribution

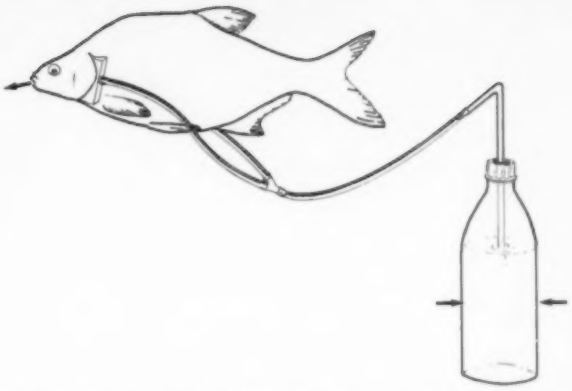

FIG. 1. Equipment used for the collection of mucus boluses from live bream using a backflow of water. A hollow perspex wedge with the shape of the opercular slit is held in place on both sides of the fish by hand and the squeeze bottle is compressed to generate a flow of water through the opercular slits and the pharyngeal cavity and out through the mouth. This was performed directly after catching.

of zooplankton in the lake appeared to be random (see also de Nie et al. 1980). Bream >15 cm FL are filter feeders at the zooplankton densities we found (Hoogenboezem et al. 1992), and the retention of particles that are larger than the mesh size of the fish (depending on the size of the fish; van den Berg et al. 1992; Hoogenboezem et al. 1993) is 100%. Furthermore, it is assumed that the fish >15 cm FL have only been filter feeding randomly (without any selection). The mesh size (MS) of the branchial sieve of bream can be calculated with the formula (Van den Berg et al. 1992)

$$MS = -0.1115 + 0.004286 \cdot FL.$$

The retention ability is given by the retention percentage for cladocerans <MS, which is calculated with the formula

$$Ret.\% = 100\% \cdot (nsb \cdot LDl)/(nlb \cdot LDs)$$

where nsb = number of cladocerans <MS that were found in the bolus, nlb = number of cladocerans >MS that were found in the bolus, LDl = lake density of cladocerans >MS, and LDs = lake density of cladocerans <MS.

Histology of the Oropharyngeal Cavity

The branchial baskets and palatal organs of some of the freshly captured bream were preserved in Bouin's fixative (Romeis 1968). Standard procedures were used for histological study (cf. Hoogenboezem et al. 1991). Sections (5 μm) were cut along the axis of the branchial arches with the palatal organ intact just above. Crossmon staining (Romeis 1968) was used on every first of five successive sections to measure the surface dimensions and to make a three-dimensional reconstruction of the total filter apparatus. Each second section was stained with Periodic Acid Schiff's (PAS) reagents (McManus and Mowry 1963) to reveal both acid and neutral mucus-producing cells. Acid mucopolysaccharides and glycoproteins were identified in each third section by a positive reaction after staining with Alcian Blue (AB) at pH 2.5 (McManus and Mowry 1963). A histochemical reaction with High Iron Diamine (HID) on every fourth section (Spicer 1965) further distinguished acid sulphomucines (positive staining) from acid sialomucines (negative staining). Subsequent staining with both AB and HID on each

fifth section allowed a comparison with the results of the individual dyes.

The size and volume of the different types of mucus-producing cells were measured according to the method of Drost and van den Boogaart (1986) on successive sections of 50 different cells of each cell type. Individual cells could be traced even though neighbouring sections of the same cell were stained differently. The density (cells per square millimetre) of the different types of mucus-producing cells was estimated on every tenth slide throughout the entire oropharyngeal cavity, taking the size of the different cell types and the thickness of the sections into account.

Histology of Mucus Boluses

Four of the preserved mucus boluses collected from bream (25, 27.5, 28, and 30 cm FL) were prepared for histological study. They were first placed in small bottles with an inlet and an outlet tube and then carefully rinsed with slowly flowing distilled water for 1 d. The material was then dehydrated by perfusion with 96% ethanol and impregnated with Technovit solution (Kulzer, Technovit 7100) without hardener. After 24 h the boluses were carefully placed in a teflon mold containing Technovit solution with hardener. Sections of 1 μm were cut on a LKB microtome with a glass knife. The sections were mounted on slides and stained using the procedure of Crossmon and with toluidine blue, PAS, and HID according to standard procedures, with the exception that the incubation periods were two to three times longer.

Results

Occurrence and Volume of Mucus Boluses

From April to November 1988, several hundred bream were caught and screened for the presence of mucus boluses in the oropharyngeal cavity. Boluses were most abundant in spring and summer, coinciding with the period of high zooplankton densities and high temperatures (Fig. 2).

In the first week of June 1988, 143 bream were caught in Lake Tjeukemeer, of which 20 were smaller than 15 cm FL, 79 were between 15 and 30 cm FL, and 44 were larger than 30 cm FL (Table 1). From the first group, all fish had a bolus present, the average volume being 0.23 mL (SD 0.16). In the intermediate group, 65% possessed a bolus with an average volume of 0.75 mL ± (SD 0.31), and in 63% of the largest fish, a mucus bolus was found with an average volume of 1.14 mL (SD 0.28) (Table 1). In all cases in which the isolated mucus was a distinct bolus, zooplankton were found inside the bolus. In approximately one third of the fish, no clear bolus was present but only a sheet of mucus. These were not investigated for the presence of zooplankton because the mucus was probably formed while the fish were being captured.

The volume of the mucus boluses increased significantly ($p < 0.001$; $Y = 0.044X - 0.34$) with the size of the fish from which they were isolated. Also, the average volume of mucus boluses in the different size classes of bream showed the same increase with fish size throughout the season (Fig. 3).

Dissection revealed that the boluses were found in the posterior part of the pharyngeal cavity, covering the fourth gill arch and the anterior part of the pharyngeal bone. In some fish, a sheet of mucus covered the entire branchial basket. The shape of the mucus boluses varied. On the ventral surface, imprints of the branchial arches were occasionally observed. Due to the soft nature of the boluses, their exact dimensions cannot be given.

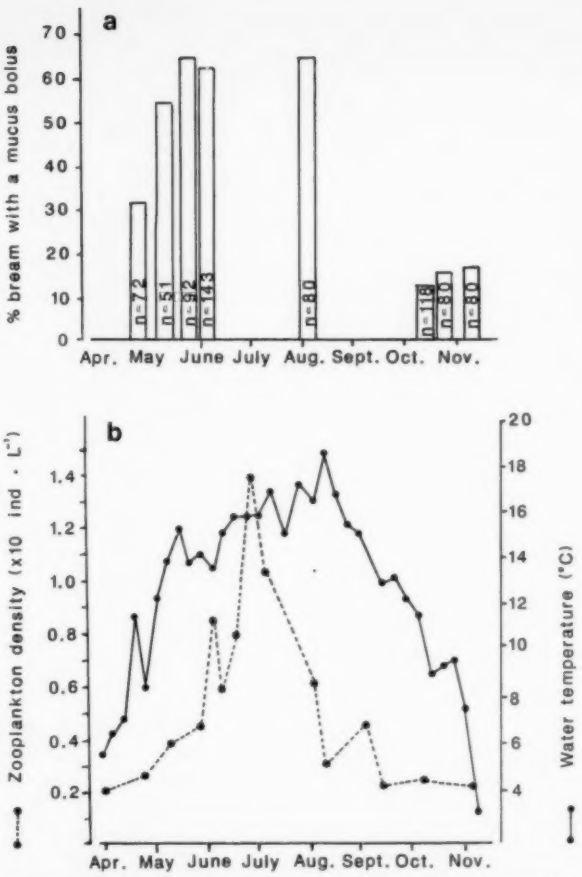

FIG. 2. (a) Percentage of bream caught in Lake Tjeukemeer during different periods in 1988 with a mucus bolus in the oropharyngeal cavity. n = total number of bream caught during a period. (b) Total zooplankton density (*Daphnia*, *Bosmina*, *Chidorus*, and copepods) and temperature in Lake Tjeukemeer during the same period. During the summer, high densities were found.

TABLE 1. Occurrence and average size (± SD) of mucus boluses in the different size classes of freshly caught bream from Lake Tjeukemeer (first week of June 1988).

Fork length (cm)	Number of fish	% with a mucus bolus	Average volume of mucus bolus (mL)
FL < 15	20	100	0.23 ± 0.16
15 < FL < 30	79	65	0.75 ± 0.31
FL > 30	44	63	1.14 ± 0.28

Contents of the Mucus Boluses

The mucus boluses usually contained cladocerans (*Daphnia* spp., *Bosmina* spp., and *Chydorus sphaericus*). Technovit microtome sections (1 μm) of a mucus bolus showed that each individual food particle was enveloped by a thin mucus layer or primary envelope. Groups of such primary envelopes were embedded in a thin mucus layer or secondary envelope (Fig. 4) and so on. Thus, a bolus is a conglomerate of small mucus envelopes. Very few algae were found in the isolated mucus, in spite of their abundance in the eutrophic lakes.

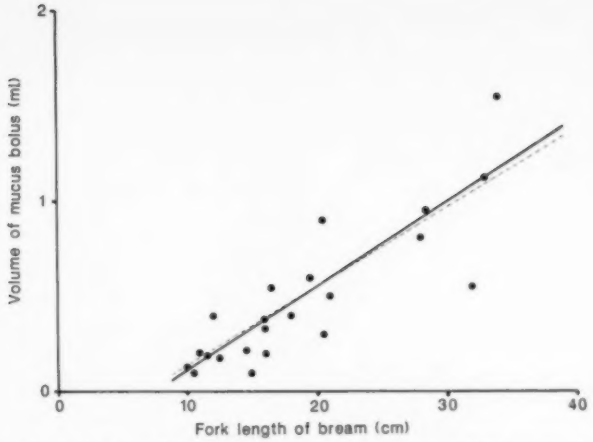

FIG. 3. Fork length versus bolus volume of bream caught in one trawl in the first week of June 1988 in Lake Tjeukemeer. The volume of the mucus boluses increases significantly ($p < 0.001$; $Y = 0.044\ X - 0.34$) with fish size. Average bolus volume in fish from different size classes from trawls throughout the season revealed the same increase with fish size (broken line).

Mucus is not only used during pelagic feeding. One trawl in Lake Langweerder Wielen in September 1988 produced 12 bream with boluses present of which 11 contained benthic cladocerans (*Alona* and *Leydigia*), sand, and debris (particle size between 0.5 and 2 mm). One bream >30 cm FL from Lake Tjeukemeer caught in June 1988 had a bolus containing approximately 50 small chironomid larvae (*Einfeldia* spp., *Polypedilum* spp., *Cladotanitarsus* spp., and *Procladius* spp.), which must be a result of benthic feeding. Therefore, we concluded that the small food particles are enveloped in mucus prior to transport during benthic feeding, as well.

Mucus Boluses and the Seasonal Activities of Bream

For each bream caught in the first week of June (when zooplankton density was high: *Daphnia* spp., 125 ind·L^{-1}; *Bosmina* spp., 530 ind·L^{-1}), we calculated the retention percentage for zooplankters that were smaller than the mesh size of the branchial sieve for that bream. The retention ratios for the small cladocerans in the group of bream >15 cm FL (that use random filter feeding at these densities, i.e. without selection (Hoogenboezem et al. 1992)) showed two distinct groups. One group had a high retention percentage (average 77% (SD 11.9)) and the other group had a low retention percentage (average 24% (SD 8.5)) (see Table 2). Fish <15 cm FL use predominantly particulate feeding (Hoogenboezem et al. 1992). No systematic differences were found in contents and morphology of the boluses collected either from live bream by backwashing or from those isolated from dissected bream.

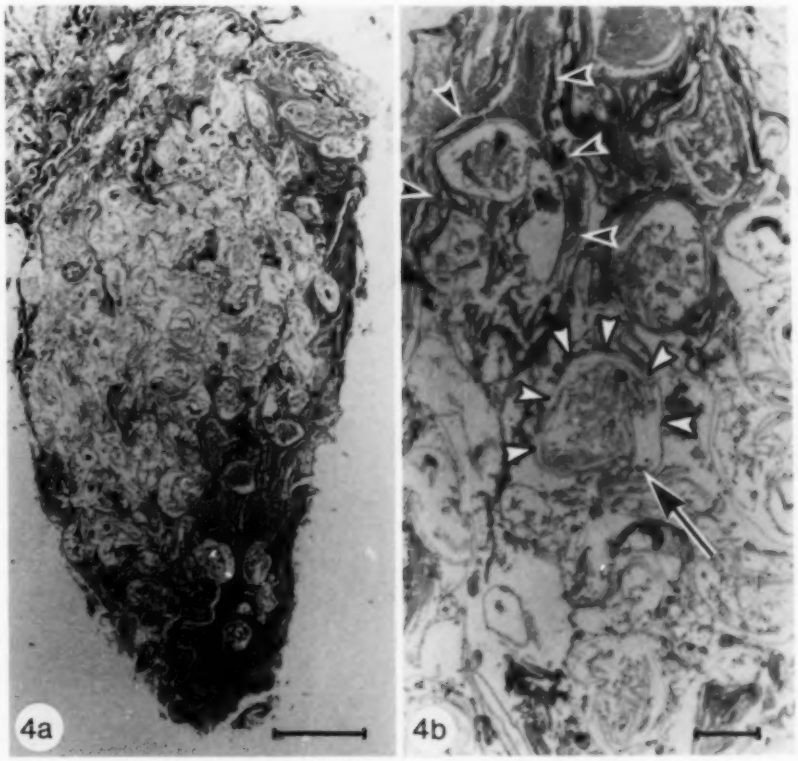

FIG. 4. (a) Section (1 μm) through a mucus bolus from a 25 cm FL fish stained with toluidine blue. Large numbers of cladocerans are enveloped in mucus. Scale bar = 1 mm. (b) Magnification of some cladocerans enveloped in mucus. The arrow indicates an individual cladoceran measuring about 0.5 mm in length. Note the primary (white-on-black arrowheads) and secondary (black-on-white arrowheads) mucus envelopes. Scale bar = 0.2 mm.

TABLE 2. Content of boluses and retention percentage for bream caught in June 1988. Of 26 bream, six had a volume of mucus without or with very few cladocerans. Six bream were smaller than 15 cm FL and had probably been foraging by particulate intake. Eight specimens (A) were considered to have foraged in the filter-feeding mode, using unreduced channels resulting in a low retention of cladocerans smaller than their mesh size. Six bream (B) had been filter-feeding with reduced channels resulting in a high retention for cladocerans smaller than their mesh size.

	Fork length (cm)	Mesh size (MS) of the branchial sieve (mm)	Number of cladocerans found in the bolus >MS	Number of cladocerans found in the bolus <MS	Retention % for cladocerans <MS	Most probable feeding mode
	14.5	0.51	0	2		Undefined
	16.0	0.57	0	0		
	20.5	0.77	0	3		
	22.0	0.83	0	0		
	25.0	0.96	0	0		
	28.5	1.11	0	9		
	10.0	0.32	6	0	0	Particulate intake
	10.5	0.34	8	0	0	
	11.0	0.36	12	1	6	
	11.5	0.38	19	2	8	
	12.0	0.40	15	24	106	
	12.5	0.42	3	0	0	
A	16.0	0.57	44	36	18	Gulping with low retention for cladocerans <MS; average 24%
	16.0	0.57	25	20	18	
	19.5	0.72	89	141	24	
	20.5	0.77	4	10	34	
	21.0	0.79	95	305	41	
	28.0	1.09	77	312	19	
	32.0	1.26	37	449	19	
	34.0	1.35	1	24	22	
B	15.0	0.53	8	32	100	Gulping with high retention for cladocerans <MS; average 77%
	16.0	0.57	4	12	67	
	16.5	0.60	106	430	77	
	18.0	0.66	71	311	76	
	19.5	0.72	154	737	71	
	33.0	1.30	3	170	70	

Morphometry of the Branchial Sieve

For a bream of 30 cm FL, the surface area of the branchial basket including the area of the palatal organ was 2750 mm^2, as calculated from three-dimensional reconstruction of 5-μm sections. The density of sulphomucine cells increased from anterior (<500·mm^{-2}) to posterior (>20 000·mm^{-2}) on each ceratobranchial and on the palatal organ above these arches (Fig. 5a and 5b). The density of the sialomucine cells was consistent over the total area of the branchial sieve (800–1200·mm^{-2}). In the posterior central sieve area (i.e. arch 4, the postlingual organ, and the corresponding area on the palatal organ just above), a very high mucus cell density (occasionally >40 000 s·mm^{-2}) was observed, consisting mostly of sulphomucine cells. On gill arches 2, 3, and 4 the density of both mucus cell types was significantly higher on the medial side of each arch than on its lateral side (Mann–Whitney U-test, $p < 0.01$; arch 2: $U = 268.5$, $n = 19$; arch 3: $U = 171.5$, $n = 15$; arch 4: $U = 134.0$, $n = 13$). There was no significant difference in the density of mucus cells on either the lateral or the medial side of the first gill arch ($U = 203$, $n = 21$) (Fig. 6).

The ratio of the total available volume of sulphomucines to that of sialomucines in the branchial sieve area is 9:1. The average volume of mucus per cell, reconstructed from serial sections, is 2.7 pL ($n = 50$) for sulphomucine and 1.4 pL ($n = 50$) for sialomucine. This gives a total intracellular mucine volume in the anterior part (70%) of the four gill arches (where individual mucus cells were easy to identify) of 27.4 μL for sulphomucine (number of cells 10.2×10^6) and 3.12 μL for sialomucine (number of cells 2.27×10^6).

Discussion

The role of mucus in the filter-feeding process of fishes has been discussed by several authors, most of whom consider mucus important for particle retention. Greenwood (1953) observed a discrepancy between the coarse architecture of the branchial sieve and the tiny phytoplankton organisms (*Melosira* spp.) ingested by *Tilapia esculenta*. He suggested that these small particles would be entangled in a copious mucus supply secreted from the epithelium of the buccal and pharyngeal cavity. He thought that the mucus–food aggregate might be prevented from escaping through the opercular slits by the gill rakers and was transported posteriorly. Mucus was supposed to be involved here in both particle retention and transport; however, it is not clear whether Greenwood (1953) actually observed these aggregates. Matthes (1963) described a possible mucus retention mechanism for *Labeo*, an African cyprinid genus. He suggested that thorough mixing in the buccal cavity of tiny food particles (e.g. diatoms) with mucus should serve to aggregate particles in larger complexes prior to filtering by the branchial sieve. No special transport or storage function was attributed to the mucus. These hypotheses have not been tested experimentally.

In view of the distribution of clavate mucus cells on the gill arches, Northcott and Beveridge (1988) assumed that in tilapia (*Oreochromis niloticus*), mucus forms a netlike structure between the arches which acts as the actual sieve. The fry (40–60 mm) of this species appeared to forage effectively on suspended bacteria (*Chromatobacterium violaceum*). Beveridge et al. (1989) assumed that the highly negatively charged mucus

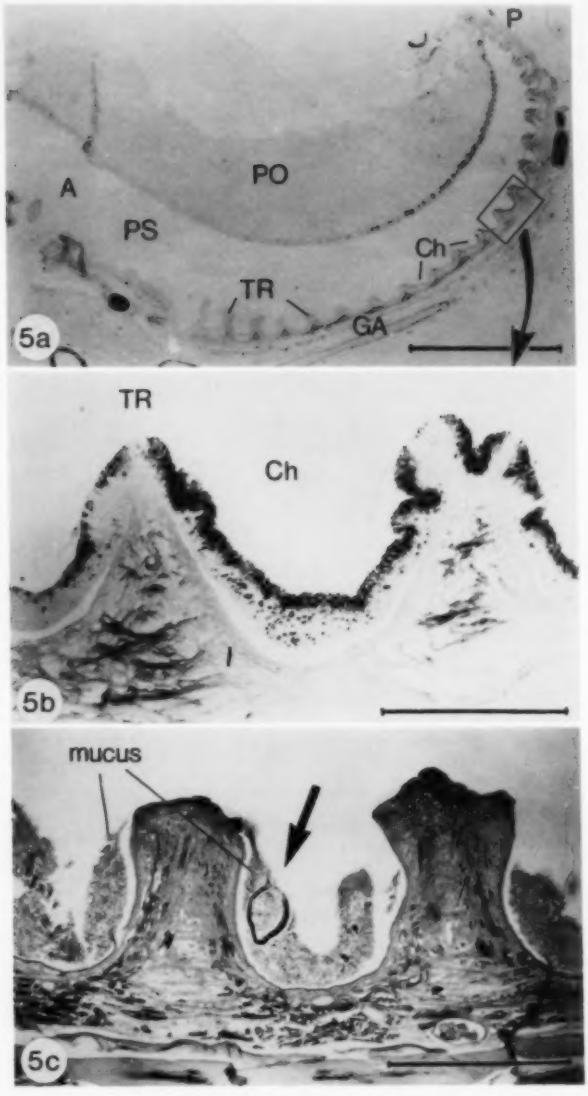

FIG. 5. (a) Sagittal section (5 μm) of gill arch 2 and the palatal organ of a 30 cm FL bream. Mucus cells are stained with High Iron Diamine (HID) and Alcian Blue (AB) (small dark spots; tissue is not counterstained). A = anterior, P = posterior, PO = palatal organ, GA = gill arch, Ch = channel, TR = transverse ridge, PS = pharyngeal slit. Note the high density of mucus cells in the walls of the channels and in the opposed palatal organ area. Scale bar = 10 mm. (b) Detail of Fig. 5a. Scale bar = 1 mm. (c) Section of a 26 cm FL bream comparable with Fig. 5b, stained after the method of Crossmon (Romeis 1968). The arrow indicates a cladoceran (*Bosmina*) in the channel wrapped in mucus. Scale bar = 1 mm.

facilitates flocculation and entrapment of these very small particles. These studies suggest a special role for mucus in filter feeding, although direct evidence such as the presence of mucus–particle aggregates or mucus boluses is lacking.

From the interesting endoscopic observation (Sanderson et al. 1991) of mucus-bound particles in live Sacramento blackfish, it cannot be concluded whether the particles were retained in mucus or if they were transported to the esophagus following mechanical retention.

Sibbing and Uribe (1985) and Sibbing et al. (1986) were, as far as we are aware, the first who suggested different roles for different mucus types in the feeding process of common carp. The roles of lubrication, reduction of water flow resistance, and protection of the epithelium were attributed to the sialomucines whereas the sulphomucines, in addition to lubrication and protection, play a role in the trapping and aggregation of food particles. They based their hypothesis on a combination of detailed histological studies and analyses of feeding mechanisms.

The presence of mucus-bound zooplankton in bream demonstrates that mucus is important in the feeding process. The question remains what the actual function of mucus is. Rubenstein and Koehl (1977) considered that a sticky filter surface may function as a retention site. This is unlikely in bream because the changing selectivity of bream in different food circumstances (Lammens 1985; Hoogenboezem et al. 1991) cannot be explained by the mechanism of a sticky filter. Furthermore, the contents of the mucus boluses we analyzed showed a different selectivity for small cladocerans between fishes (e.g. 24 and 77%; Table 2). Also, planktonic algae were absent in the bolus whereas huge numbers of algae were present in the environment, and benthic feeding bream had very few debris particles smaller than the mesh size of their sieve in their boluses. Because these particles are too small to be retained mechanically, they flow through the sieve without stimulating mucus excretion and without sticking to earlier excreted, locally available mucus. Most probably the mucus serves to transport relatively small particles from the retention sites in the channels to the esophagus.

Model

We hypothesize the role of mucus in filter-feeding of bream in three steps: (1) wrapping, (2) accumulating, and (3) storage and transport. Water containing food particles is engulfed, and the particles are retained mechanically in the channels of the gill arches (Hoogenboezem et al. 1991), without the use of mucus.

1. Wrapping

The contact between particle and channel surface stimulates mucine secretion around the prey, wrapping it in a primary mucus sheet. The mucus-enveloped particles are then supposed to behave as sticky particles, ready to be manipulated and transported.

2. Accumulating

After a number of gulps, when clogging starts to impede the filter action and reduce its capacity per unit time, these sticky particles are flushed back from the sieve to the oral cavity by a closed protrusion movement. During closed protrusion the buccal cavity is expanded while the lips are kept closed, causing a water flow from the open opercular slits into the oral cavity in an anterior direction (Sibbing et al. 1986; Sibbing 1991). During compression of the buccal cavity, different particles wrapped in a primary sheet of mucus stick together and form a small mucus bolus that is transported to the posterior part of the pharyngeal chamber (between the palatal and postlingual organs) and sticks to the abundant mucus in that area or to a previously formed bolus. This again will stimulate mucine secretion in this area so that the total aggregate is enveloped in mucus once again (second sheet).

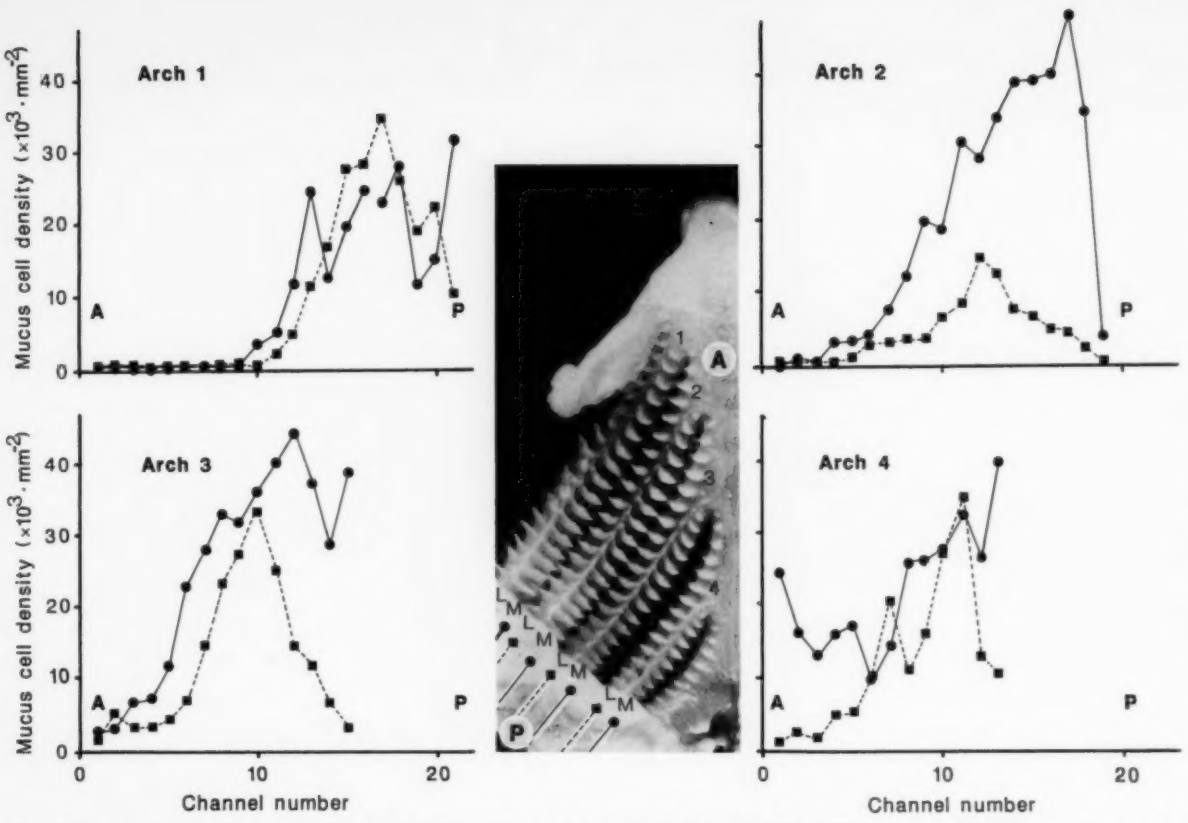

FIG. 6. Density of mucus-producing cells found in different parts of the branchial sieve of a 30 cm FL bream. The densities are determined in the subsequent channels on the four gill arches (1–4). Only the channels that have an opposite channel on the same gill arch are plotted. Note the distinct increase in density from anterior to posterior on the branchial sieve and also the higher numbers on the medial side of gill arches 2, 3, and 4 compared with the lateral side. A = anterior, P = posterior, L = lateral (broken line), *m* = medial (solid line).

3. Storage and transport

For several of these wrapping and accumulating cycles the bolus remains stored in the central posterior area of the pharyngeal cavity and will grow larger. By muscular peristalsis of the palatal and postlingual organs the large bolus is transported towards the esophagus. Through pharyngeal jaw movement, squeezing of the bolus and particles will also occur. The bolus will be swallowed subsequently (c.f. Sibbing et al. 1986).

Evidence for the first step is provided by microscopic observations of the Technovit slides of a mucus bolus that show that each single prey item in the bolus is enveloped in mucus (Fig. 4) and by the microscopic observation of a section of a gill arch with a zooplankter wrapped in mucus in a channel (Fig. 5c). The first step of this model is consistent with an adjustable filter system (Hoogenboezem et al. 1990, 1991), where channels are considered to be the retention sites for prey, with the medial channels used particularly for the small prey. The higher mucus cell density in the medial channels, as observed in this study (Fig. 6), is consistent with this hypothesis. It also provides important additional support for this explanation. Retained particles may stimulate these cells to discharge mucus by direct contact. Discharge of mucus may also be aided by muscle activity. The cushionlike structure of the raker contains a complex network of muscle fibers, the musculus constrictor canalis interbranchiospinalis (Hoogenboezem et al. 1991). Contraction of this muscle may increase mucus discharge, forming a mucus web, e.g. prior to closed protrusion movements.

The second step in the process is supported by our observations of distinct subunits in the bolus, seen microscopically. Further, the enormous density of mucus cells in the posterior part of the pharyngeal cavity would produce the mucus required for accumulation, growth, and storage of small aggregates of this material in this area. Frequent closed protrusion movements during filter feeding (also in common carp, c.f. Sibbing et al. 1986, fig. 4a) further support these ideas.

The third step is confirmed by the fact that foraging bream store strained zooplankton for quite some time in the pharyngeal cavity. Particle counts per bolus revealed up to 150 prey items larger than the mesh size of a 19.5 cm FL bream (Table 2), which means that a volume of 1.05 L of water should have been filtered. Bream of this length are able to process approximately 4.5 mL of water per snap (mouth volume = $6 \times 10^{-6} \cdot FL^3$, Hoogenboezem et al. 1992); thus, over 220 snaps were taken to collect and retain these particles. The snap frequency of bream of this size class is estimated as approximately 1.2 snaps·s^{-1} (Hoogenboezem et al. 1992); therefore, it took over 3 min to constitute this bolus. Because bolus formation requires several minutes, the actual volume and contents of the bolus of a fish caught at a certain moment may vary from a low to a high value (the latter just before swallowing). The ratio between large and small prey items is not necessarily influenced in this time span, assuming random filter feeding.

Video recordings of bream foraging in an aquarium containing a zooplankton composition comparable with Lake Tjeukemeer revealed closed protrusion movements about every 10 s, which is also confirmed by earlier X-ray films of bream foraging at high zooplankton densities (>100 ind·L^{-1}, Hoogenboezem et al. 1990). We therefore must address the question whether mucine production is high enough to produce a sufficient volume of mucus every 10 s.

The volumes of intracellular mucines in bream were between 1.4 and 2.7 pL. According to Verdugo (1984), mucines have a swell ratio of 10^2–10^3. Sheehan and Carlstedt (1984) reported that a concentration of mucin of 0.5 mg·mL^{-1} is sufficient to start physical entanglement (i.e. mucine molecules begin to interpenetrate to form a mucus web). A single cell in the mammalian respiratory tract is able to produce 9–18 nL·min^{-1} (Widdicombe 1988), which means a production of at least 9–18 pL mucine·$cell^{-1}$·min^{-1}. If we use this number and a swell factor of 10^3 for the mucus cells of bream, the total production of mucines in bream can be calculated. The number of mucus-producing cells in the anterior 70% of the branchial sieve (i.e. the actual retention site; about 2000 mm^2) is sufficient to produce a continuous mucus layer 35 μm thick over this area every 10 s! The actual intracellular volume of mucus cells in bream is probably underestimated because measurements were carried out on fixed material causing a shrinkage of approximately 30% (Drost and van den Boogaart 1986). On the other hand, mucine production in poikilotherm fishes will be lower compared with that in homeotherm mammals. Still, we believe that the above-mentioned level of mucus production is sufficient to support the hypothesized feeding mechanism.

All the above data fit the picture of a fish retaining small food particles by sieving, later covering them with mucus and accumulating these aggregates in larger lumps prior to deglutition. This mucus-based food aggregation mechanism is convergent with filtering systems found in early agnathe vertebrates (Lessertisseur and Robineau 1969) and in recent cephalochordates. Apparently the abundance of small but highly nutritive particles in the water column provides such an important food source that new mechanisms have evolved to harvest them. In bream, and perhaps in cyprinids in general, the combination of the use of mucus with an adjustable branchial filter provides a successful way to expand as a species in man-made eutrophic environments.

Acknowledgements

We thank J. W. M. Osse, F. A. Sibbing (Wageningen), E. H. R. R. Lammens, and J. Vijverberg (Oosterzee) for their stimulating discussions and critical remarks on the manuscript. B. van den Bergh and T. Machielsen are kindly acknowledged for their help in collecting ecological and histological data. We also thank K. Abbot for her assistance in improving the English. The investigation was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for Scientific Research (NWO Project 811-437-241B).

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Hybridization with Domesticated Rainbow Trout (*Oncorhynchus mykiss*) Reduces Seasonal Variation in Growth of Steelhead Trout (*O. mykiss*)

Jörgen I. Johnsson,[1] W. Craig Clarke, and Ruth E. Withler

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Johnsson, J. I., W. C. Clarke, and R. E. Withler. 1993. Hybridization with domesticated rainbow rout (*Oncorhynchus mykiss*) reduces seasonal variation in growth of steelhead trout (*O. mykiss*). Can. J. Fish. Aquat. Sci. 50: 480–487.

We tested the hypothesis that hybridization with domesticated rainbow trout (*Oncorhynchus mykiss*) reduces the seasonal variation in growth in both fresh and salt water in anadromous steelhead trout (*O. mykiss*). The seasonal variation in freshwater growth was more pronounced in the steelhead trout than in the hybrid and domesticated trout. Consequently, differences in growth rate among groups were considerably higher during winter than in fall or spring. We suggest that high feeding activity during unfavourable winter conditions in natural waters is maladaptive, but is not selected against in protected hatchery environments with a continuous food supply. This may lead to genetic divergence in feeding physiology and/or behaviour between steelhead trout and domesticated rainbow trout. Growth rates in experimental groups transferred to seawater in December relative to groups transferred in April were faster in rainbow and hybrid trout than in steelhead trout. The results support the hypothesis that seasonal variation in seawater growth in steelhead trout is reduced by crossing with domesticated rainbow trout.

Nous avons vérifié l'hypothèse à l'effet que l'hybridation avec la truite arc-en-ciel (*Oncorhynchus mykiss*) d'élevage atténue les variations saisonnières de croissance tant chez la steelhead (*O. mykiss*) d'eau douce que la steelhead anadrome d'eau salée. Les variations saisonnières de la croissance en eau douce sont plus prononcées chez la steelhead qu'elles ne le sont chez son hybride et la truite d'élevage. Par conséquent, les différences du taux de croissance qui sont observées entre les groupes sont considérablement supérieures l'hiver à ce qu'elles sont en automne ou au printemps. Nous avançons l'hypothèse à l'effet qu'une alimentation intense des sujets dans des eaux naturelles sous les conditions défavorables de l'hiver est une maladaptation, mais qui ne joue pas dans l'environnement protégé des piscicultures où une alimentation régulière est assurée. Cela peut conduire à une divergence génétique dans le comportement et(ou) la physiologie alimentaire de la steelhead et de la truite arc-en-ciel d'élevage. Chez des groupes transférés dans l'eau de mer en décembre, comparativement à des groupes transférés en avril, la croissance est la plus rapide chez la truite arc-en-ciel et la truite hybride que chez la steelhead. Ces résultats viennent confirmer l'hypothèse selon laquelle la variation saisonnière de la croissance en mer de la steelhead est réduite par l'hybridation aec la truite arc-en-ciel d'élevage.

Received January 16, 1992
Accepted September 14, 1992
(JB370)

Reçu le 16 janvier 1992
Accepté le 14 septembre 1992

Natural rainbow trout (*Oncorhynchus mykiss*) populations exhibit a large amount of phenotypic variation, including both anadromous and freshwater resident life history patterns (Hershberger 1992). The anadromous form, termed the steelhead trout, generally spends 1–3 yr in fresh water before smoltification (Folmar and Dickhoff 1980) and seaward migration during late spring (Ewing et al. 1984). Seawater adaptability increases with size in steelhead trout (Houston 1961; Conte and Wagner 1965; Clarke 1982). However, smoltification and seawater adaptability are also controlled by an endogenous cycle (Wagner 1974) and synchronized by environmental cues, primarily temperature (Zaugg and Wagner 1973; Wagner 1974) and photoperiod (Wagner 1974; Johnsson and Clarke 1988).

The rainbow trout has been domesticated since the 1870s (Hershberger 1992). Selective breeding has traditionally favoured stocks with rapid growth rates and high food conversion efficiency in fresh water (Madsen and Naamansen 1989). Selection for improvement of these commercial traits has been very successful (Gall and Huang 1988; Gjerde and Schaeffer 1989). In recent years, seawater rearing of domesticated rainbow trout has expanded at an increasing rate (Gjerde 1989; Madsen and Naamansen 1989). The seasonal variation in seawater adaptability seems to be diminished in domesticated rainbow trout compared with anadromous steelhead trout (Boeuf and Harache 1982; Madsen and Naamansen 1989). Johnsson and Clarke (1988) found that simulated spring daylength exposure in January accelerated the development of salinity tolerance and silvering and decreased condition factor in anadromous steelhead trout, while these smoltification-associated changes did not occur in domesticated rainbow trout. Hence, wild and domestic types seem to differ in the phenotypic expression of traits associated with growth and seawater adaptability. However, studies of the genetic control of these traits have not been reported.

The purpose of this study was to test the hypothesis that hybridization with domesticated rainbow trout reduces seasonal

[1]Present address: Department of Zoology, University of Göteborg, Medicinaregatan 18, S-413 90 Göteborg, Sweden.

variation in growth of steelhead trout both in fresh and in salt water.

Methods

Study Populations

Wild steelhead trout were obtained from the winter-run population in the Big Qualicum River (one male, six females) and the summer-run population in the Puntledge River (eight males, three females). Both rivers are situated on the east coast of Vancouver Island, approximately 50 km apart. Domesticated rainbow trout were obtained at HyWave Sea Farm in Prince Rupert from a stock that has been domesticated for at least 20 yr. On April 6, half of the eggs from each wild steelhead female were fertilized by a wild male and the other half by one of the nine domesticated males, creating nine pairs of half-sib families. Each male was used only once, except one domesticated male, which was also used to fertilize the single rainbow trout female. Eggs obtained from additional domesticated rainbow trout females were not fertile.

Husbandry

The experimental crosses were produced at the Rosewall Creek hatchery, where the eggs were incubated (mean water temperature 8.0°C) to the eyed stage. On May 3 the eyed eggs were transported to the Pacific Biological Station for further incubation (mean temperature 10.9°C). The eggs hatched on May 16–17 and on June 9 the swim-up fry from each family were transferred to net cages in separate freshwater tanks. After a few days the cages were removed and the families were subsequently reared in 197-L fibreglass tanks at ambient water temperature (see Fig. 1). Throughout the experiments, the fish were fed a commercial salmon diet at 1.2–1.3 times the rates recommended by the manufacturers. Food was provided continuously during daylight hours using automatic disc feeders. Natural photoperiod was simulated using EC 71 electronic timers and the light intensity was 150–180 lx 10 cm over the water surface. On July 18 the fish densities in all tanks were adjusted to 1.7 g·L water^{-1}.

Growth Experiment

On August 28–29, 40 fish from each family were individually tagged with passive integrated transponder tags (model No. TX 1400, Destron Identification Devices Inc.). On August 31, four randomly chosen tagged individuals from each of the nine steelhead families were pooled in one of 10 tanks creating 10 replicate tanks, each with 36 individuals. The nine hyrid families were pooled the same way whereas the single family of domesticated rainbow trout was split into four tanks, each with 15 individuals.

On December 8, five of the 10 replicate tanks for each type (two for the domesticated family) were switched from fresh water to ambient 30% seawater by turning off the freshwater supply and turning on seawater valves. The salinity change was completed in 30 min. On January 6, 1990, the density in each tank was reduced 25% by removing one randomly selected fish from each family (nine fish per tank). On April 23 the remaining freshwater tanks were switched over to seawater whereafter all experimental fish were reared in seawater until the termination of the experiment on May 24, 1990. During the experiment, weight and fork length were measured for each individual at regular intervals (Fig. 1).

Analysis of Variance

Analyses of variance were conducted only on the 18 steelhead and hybrid half-sib families because the single domesticated rainbow trout family could not be included in the genetic model. Experimental data were subjected to GLM analysis of variance (SAS Institute Inc. 1985). Two models were used for growth analysis:

Model A, for freshwater growth, September 8 – December 6:

$$Y_{ijkl} = \mu + M_i + F_j + MF_{ij} + R(M)_{k(i)} + e_{ijkl}$$

Model B, for growth in fresh and seawater, December 8 – April 15 and April 15 – May 24:

$$Y_{ijklm} = \mu + M_i + W_j + F_k + MW_{ij} + MF_{ik} + WF_{jk} + R(MW)_{l(ij)} + e_{ijklm}$$

where Y_{ijkl} was the growth of each individual, μ was the overall mean, M_i was the fixed effect of the ith male parent life history type (rainbow or steelhead), W_j was the fixed effect of the jth environment (fresh or seawater), F_k was the random effect of the kth female parent, MW_{ij} was the fixed effect of the male type by environment interaction, MF_{ik} was the random effect of the male type by female interaction, WF_{jk} was the random effect of the environment by female interaction, $R(MW)_{l(ij)}$ was the random effect of the lth replicate tank within male type by environment interaction, and e_{ijklm} was the random error.

The calculations of F and df values for the variance components were made according to Satterthwaite (1946). Two measurements of growth were used for the analysis: specific growth rate (Ricker 1979) calculated as $G = \ln(Wt_1/Wt_0)\cdot 100\cdot d^{-1}$ and the coefficient of growth (Iwama and Tautz 1981) computed as $GC = Wt_1^{0.333}\cdot Wt_0^{-0.333} + (T\cdot 1000^{-1})d$, where Wt_0 and Wt_1 were the initial and final wet weights for the period, T was the average temperature (degrees Celsius), and d was the time in days. The GC contains adjustment factors for temperature and size effects, thereby allowing comparison of growth among different water temperatures and among fish of differing initial size. Condition factor (CF) was computed as $100\cdot Wt\cdot L^{-3}$ where Wt was weight (grams) and L was fork length (centimetres).

Results

Early Development

Figure 1 shows the growth trends among the experimental groups during the experiment. Five days after ponding, on June 14, the mean weight of the hybrid trout was significantly higher than that of the steelhead trout ($F_{1,118} = 6.9$, $p < 0.01$). At 0.06 g, the domesticated rainbow fry were only one third as large as the steelhead (0.18 g) and hybrid fry (0.19 g). At this time, the relative yolk remains (yolk weight/body weight) in steelhead trout were on average 0.6%, compared with 0.4% in the hybrid trout ($F_{1,118} = 5.5$, $p < 0.05$), indicating a higher rate of yolk sac absorption in the hybrids. By August 8, the rapidly growing rainbow trout fry were heavier than either the hybrid or the steelhead trout.

Growth in Freshwater

Mean specific growth rate (G) and coefficient of growth (GC) of the experimental groups in fresh water from September 8 to December 6 are presented in Fig. 2. For both G and GC, the rainbow trout had the highest rate, followed by the hybrid trout and the steelhead trout. The among-group differences in GC

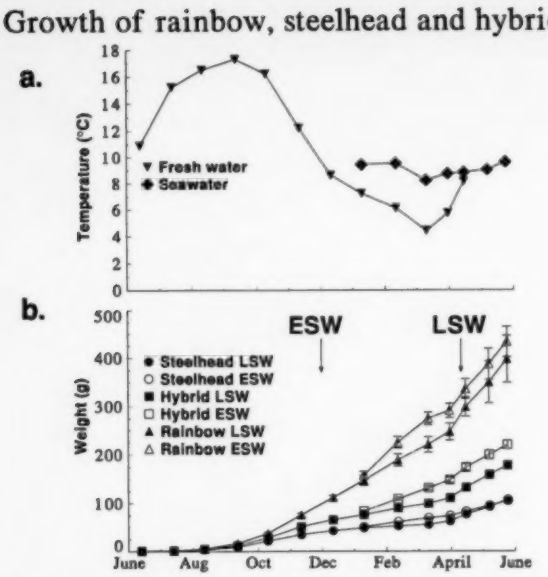

FIG. 1. (a) Temperatures in fresh and seawater during the growth experiments. (b) Weight of steelhead, hybrid, and domesticated rainbow trout in a growth experiment in fresh and seawater. ESW denotes groups transferred to seawater on December 8. LSW denotes groups transferred to seawater on April 23. Vertical bars denote standard errors of mean weights.

were greater than those for G. The effect of male parent type was significant on both G ($F_{1,10} = 7.4$, $p < 0.05$) and GC ($F_{1,10} = 23.1$, $p < 0.001$). There was no significant effect of female parent on G ($F_{8,8} = 2.6$, $p > 0.05$) or GC ($F_{8,8} = 3.0$, $p > 0.05$), but the interaction between male type and female parent was highly significant for both G ($F_{8,669} = 9.1$, $p < 0.001$) and GC ($F_{8,669} = 9.4$, $p < 0.001$). There was also a significant effect of replicate tank on both growth measures during this period ($F_{18,669} = 2.1$, $p < 0.01$ for both G and GC). The size adjustments in the GC formula increased the significance of the difference between rainbow and steelhead males on freshwater growth rate, but did not eliminate the male type by female interaction.

Figure 3 presents seasonal variation of the GC in fresh water for the experimental groups. The growth of the groups diverged from October to February, whereafter it gradually converged again from February to April. Despite the linear temperature-compensating factor in the GC formula, there was a threefold drop in GC in the steelhead trout from October to February corresponding to a temperature drop from 16 to 4°C (see Fig. 1), whereafter GC increased during spring. In contrast, the domesticated rainbow trout did not show any tendency towards reduced GC during winter. The hybrids were intermediate, exhibiting a reduction in GC during the winter which was less pronounced than in the steelhead (Fig. 3).

Growth in Fresh and Seawater

Growth (G and GC) in fresh and seawater from December 8 to April 15 is presented in Fig. 4. For steelhead and rainbow trout, specific growth rates were similar in fresh and seawater, while the hybrid trout grew relatively faster in seawater (Fig. 4a). However, when an adjustment was made for the higher mean temperature in seawater (8.9°C) compared with fresh water (6.3°C), the GC was higher in fresh water compared with seawater for steelhead and rainbow trout, while the hybrid trout showed similar growth in fresh and seawater (Fig. 4b).

The effect of male parent type was again highly significant on both G ($F_{1,9} = 16.7$, $p < 0.01$) and GC ($F_{1,16} = 29.6$, $p < 0.001$) whereas there was no significant effect of female parent on either growth measure ($F_{8,10} = 0.5$, $p > 0.05$ and $F_{8,11} = 0.8$, $p > 0.05$, respectively). The effect of environment on G was highly significant ($F_{1,9} = 20.0$, $p < 0.01$) whereas there was no significant effect on GC ($F_{1,12} = 3.4$, $p > 0.05$). This indicates that the difference in temperature,

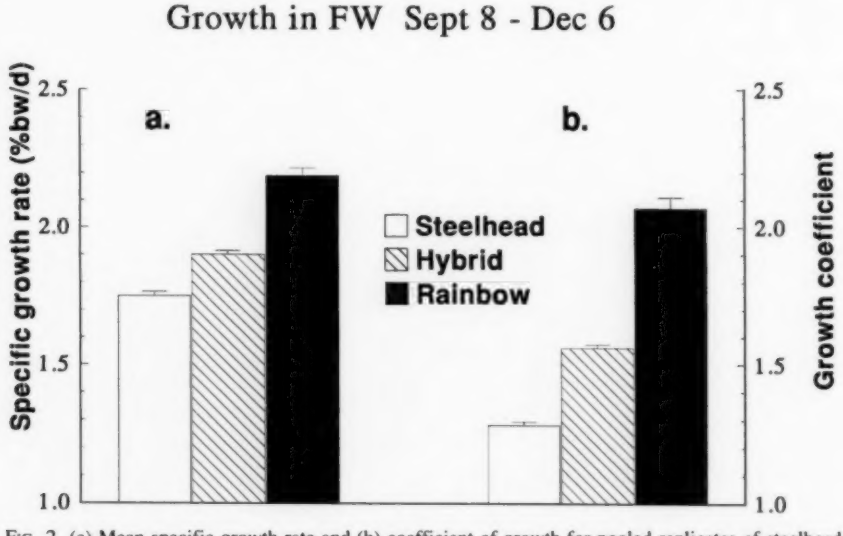

FIG. 2. (a) Mean specific growth rate and (b) coefficient of growth for pooled replicates of steelhead ($n = 353$), hybrid ($n = 353$), and domesticated rainbow trout ($n = 60$) from September 8 to December 6 in fresh water. Mean water temperature was 12.3°C. Vertical bars denote standard errors of mean growth rates.

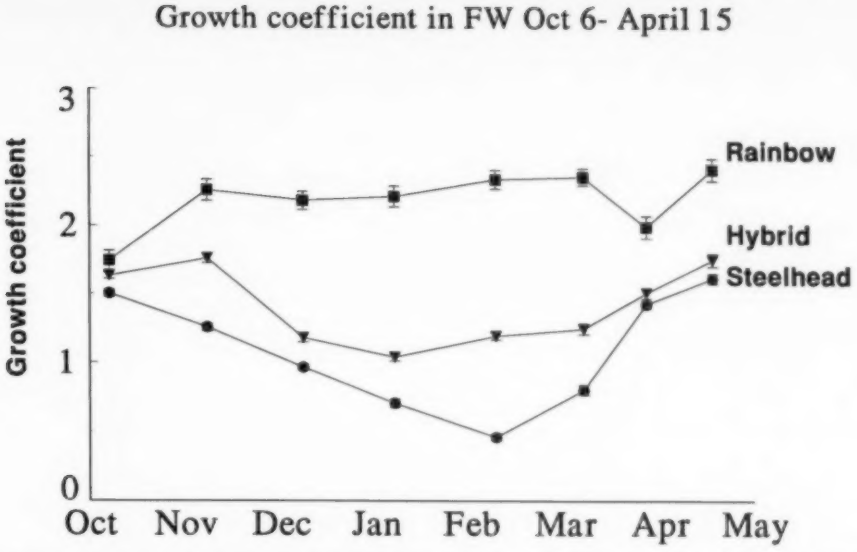

FIG. 3. Variation in the coefficient of growth (Iwama and Tautz 1981) in fresh water from October 6, 1989, to April 15, 1990, for pooled replicates of steelhead, hybrid, and domesticated rainbow trout. Vertical bars denote standard errors of group means. Initial sample sizes: $n = 358$ for steelhead and hybrid trout and $n = 60$ for rainbow trout. For water temperatures, see Fig. 1.

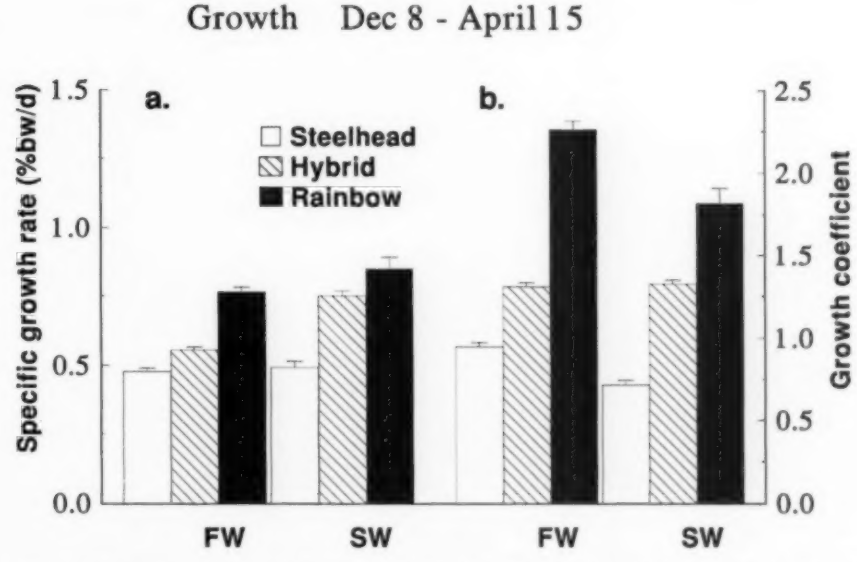

FIG. 4. (a) Mean specific growth rate and (b) coefficient of growth for pooled replicates of steelhead ($n = 240$), hybrid ($n = 272$), and domesticated rainbow trout ($n = 55$) in fresh (FW) and seawater (SW) from December 8 to April 15. Mean temperature was 6.3°C in fresh water and 8.9°C in seawater. Vertical bars denote standard errors of group means.

which is adjusted for in the calculation of GC, was the primary reason for the faster growth observed in seawater than in fresh water during this period. There was, however, a highly significant male parent type by environment interaction for both G ($F_{1,15} = 25.6$, $p < 0.001$) and GC ($F_{1,15} = 13.2$, $p < 0.01$), which reflected the relatively faster seawater growth of the hybrid trout than of the steelhead trout. For both G and GC, there were also significant effects of the male type by female interaction ($F_{8,466} = 6.3$, $p < 0.001$ and $F_{8,466} = 7.7$, $p < 0.001$, respectively) and the environment by female interaction ($F_{8,466} = 2.1$, $p < 0.05$ and $F_{8,466} = 2.9$, $p < 0.01$, respectively). There was no significant effect of replicate tank

on growth during this period ($F_{15,466} = 1.5$, $p > 0.05$ for G and GC).

Growth in Seawater

Figure 5 shows the seawater growth of groups transferred to seawater either early (ESW) or late (LSW) from April 15 to the termination of the experiment on May 24. The LSW groups grew significantly faster than the ESW groups ($F_{1,10} = 17.4$, $p < 0.01$ for G and $F_{1,11} = 11.0$, $p < 0.01$ for GC). The effect of male type was less pronounced on growth in this period ($F_{1,12} = 2.7$, $p > 0.05$ for G and $F_{1,12} = 4.3$, $p = 0.06$ for GC), but relatively better growth of LSW steelhead compared with LSW hybrid trout resulted in a significant male type by environment interaction for both G ($F_{1,15} = 5.3$, $p < 0.05$) and GC ($F_{1,15} = 5.9$, $p < 0.05$). There was no effect of the time of seawater entry on growth in the rainbow trout.

For both G and GC, there was no significant effect of female parent ($F_{8,10} = 0.7$, $p > 0.05$ and $F_{8,10} = 0.8$, respectively) in this growth period, but there were significant male type by female parent ($F_{8,462} = 1.9$, $p = 0.06$ and $F_{8,462} = 2.2$, $p < 0.05$, respectively) and environment by female parent ($F_{8,462} = 6.3$, $p < 0.001$ and $F_{8,462} = 4.9$, $p < 0.001$, respectively) interactions. There was a significant effect of replicate tank on both G ($F_{15,462} = 2.2$, $p < 0.01$) and GC ($F_{15,462} = 1.9$, $p < 0.05$).

Condition Factor

Figure 6 shows the CF for the experimental groups during the experiment. The CF was consistently highest in the domesticated rainbow trout, intermediate in the hybrids, and lowest in the steelhead trout. For both steelhead and hybrid trout, CF decreased in fresh water fromSeptember to March, but remained virtually unchanged in the rainbow trout. Throughout the experiment, there was a highly significant effect of male type (e.g. May 24, $F_{1,11} = 75.0$, $p < 0.001$) and male type by female parent interaction (e.g. May 24, $F_{8,462} = 7.8$, $p < 0.001$).

Precocious Sexual Maturation and Mortality

The incidence of precocious sexual maturation was 1.7% in the rainbow trout, 2.5% in the hybrid trout, and 0.3% in the steelhead trout. There was no "natural" mortality[2] in fresh water during the experiment. In ESW groups, the mortality in seawater was 5.0% for steelhead trout, 2.3% for hybrid trout (including one ripe male), and 3.3% for rainbow trout.

Discussion

In this study, we demonstrated that hybridization with domesticated rainbow trout reduces the seasonal variation in growth of anadromous steelhead trout. The hybrid trout grew faster than the steelhead trout in both fresh and seawater and were less seasonally variable in growth and in the ability to adapt to seawater. The lack of environmental influence on growth and smoltification in the rainbow trout maintained as a control may have been characteristic of the unknown natural rainbow trout population from which the domesticated population was derived or, more likely, may have resulted from

[2]Interrupted tank flows caused mortalities in three experimental tanks during the experiment. On January 13, all 36 steelhead trout died in a seawater replicate tank, on April 27, three rainbow trout died in a LSW-tank, and on May 5, six rainbow trout died in a ESW tank. The surviving rainbow trout in the affected tanks were not included in subsequent analyses.

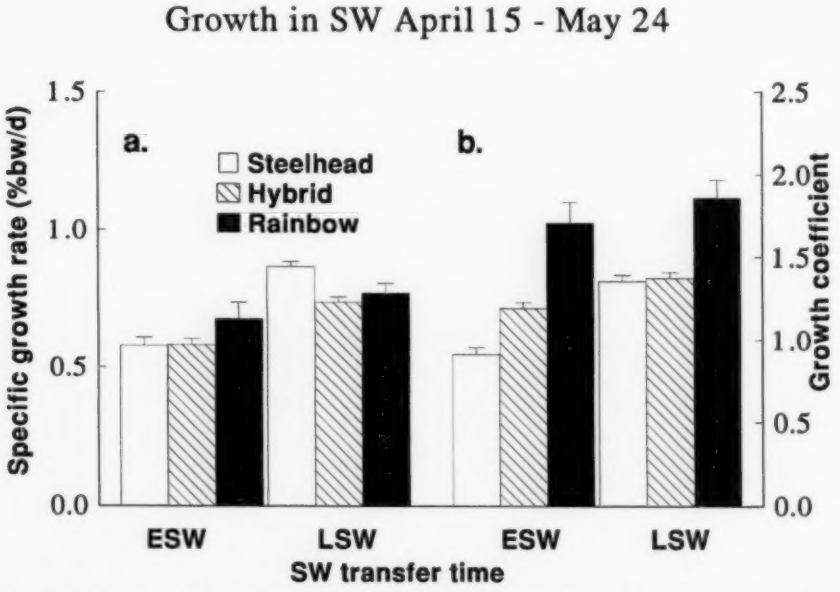

FIG. 5. (a) Mean specific growth rate and (b) coefficient of growth for pooled replicates of steelhead ($n = 235$), hybrid ($n = 270$), and domesticated rainbow trout ($n = 27$) from April 15 to May 24. ESW denotes fish transferred to seawater on December 8; LSW denotes fish transferred to seawater on April 23. Mean temperature was 9.3°C for the early group and 9.4°C for the late group. Vertical bars denote standard errors of group means.

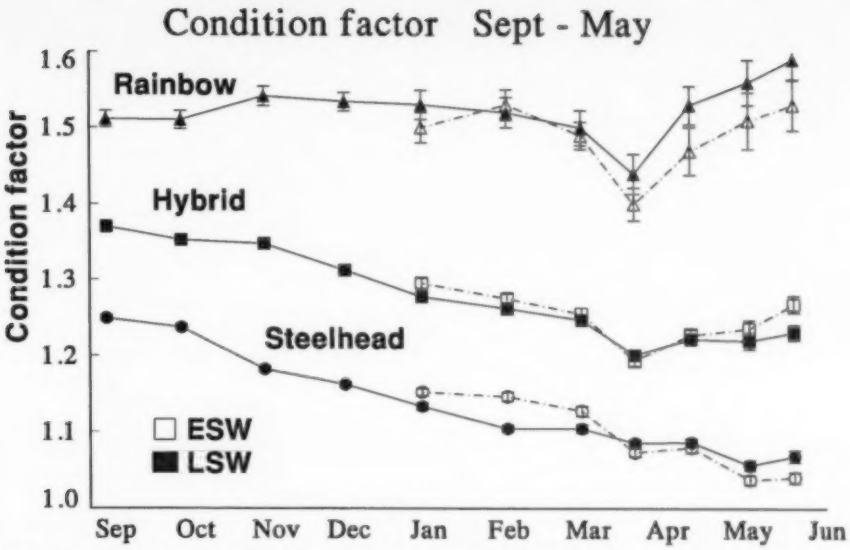

FIG. 6. Seasonal changes in CF for ESW (December 8) and LSW (April 23) transferred pooled replicates of steelhead (n = 358), hybrid (n = 358), and domesticated rainbow trout (n = 60). Vertical bars denote standard errors of group means; error bars smaller than group symbols are hidden. For water temperatures, see Fig. 1.

artificial selection during domestication. It seems probable that the highest rate of yolk sac absorption and weight at first feeding in the hybrid trout compared with the steelhead trout resulted from a correlated response in the domesticated rainbow trout (and hybrids) to selection for high growth rates at later developmental stages because weights at different ages may be genetically correlated (McKay et al. 1986).

In addition to a strong effect of male type (steelhead or rainbow) on growth of trout in this study, there was a strong genotype by environment interaction between male type and winter (December–April) environment (fresh or seawater) that affected winter growth and persisted into saltwater growth during April and May. Thus, there was a strong negative effect of early (December) tansfer to seawater on growth in steelhead but not in hybrid trout. Even after all fish were in seawater (April–May), the ESW steelhead showed a greater reduction in growth than did the ESW hybrid trout relative to the corresponding LSW groups.

There was no significant effect of female parent observed on growth during this study, but the significant male type by female parent interaction effects throughout the study indicated that the relative growth performance of progeny sired by rainbow and steelhead males differed among females. This source of variation includes variability due to individual males within each male type (because each female was crossed with a different rainbow and steelhead male), as well as nonadditive genetic effects (dominance, epistasis) on growth. The fact that the steelhead male and female parents used in this study were drawn from two populations may have increased the variability in growth performance among steelhead males and among females, as well as increasing the nonadditive genetic effects. However, for growth over most time periods, the variability introduced by these factors was not sufficient to obscure the significant differences in performance between progeny sired by rainbow and steelhead males.

There was also a significant interaction between female parent and environment that indicated that the relative performance of progeny in fresh and seawater differed among females, perhaps due to nonadditive genetic variability. The significant effect of replicate tanks on growth during part of the experiment indicated that the environment was not entirely homogeneous, although it is also possible that some variability between replicates reflected sampling variance resulting from the small number of fish representing each family in each tank. Among the experimental groups, the coefficients of growth (Iwama and Tautz 1981) diverged from October to February and converged again from February to April. This occurred because growth of the steelhead trout and, to a lesser extent, of the hybrid trout decreased over winter more than expected on the basis of water temperature alone. A possible explanation for the great reduction in winter growth of steelhead is that high feeding activity is maladaptive during unfavourable winter conditions in natural habitats (Metcalfe et al. 1988), but is not selected against in protected hatchery environments with a continuous food supply. Under natural conditions, the growth rates of juveniles are often below maximum levels, indicating that there are costs of growing, which may result in increased mortality (Sibly and Calow 1986). Positive correlations between growth and mortality rates have been described in fishes (Beverton and Holt 1959) and it is reasonable to suppose that the cost of growing is very high under harsh winter conditions in trout streams. Chapman and Bjornn (1969) found that 0 age steelhead trout hid in the river substrate in winter, a behaviour that was negatively correlated with water temperature.

The steelhead trout in our study received food in excess of satiation but growth virtually ceased during January and February, which suggests that feeding activity may be a genetically controlled trait with limited phenotypic plasticity. Lack of predation and an abundant food supply in the domesticated rainbow population may have resulted in a loss of the genetically

controlled seasonal feeding behaviour, with the differing seasonal growth patterns between steelhead and rainbow exemplifying divergent evolution of reaction norms (Stearns 1989). The steelhead and hybrid trout of this study also differed in their foraging behaviour during September (Johnsson and Abrahams 1991). The juvenile hybrid trout were significantly more willing to forage under risk of predation than were steelhead trout of similar size. It is likely that selection for rapid growth in domesticated rainbow trout has altered a variety of physiological and behavioral adaptations present in wild trout. Similar changes may occur inadvertently in hatcheries that produce juvenile salmonids for release to supplement natural production (Swain and Riddell 1990; Riddell and Swain 1991). Consequently, interbreeding between escaped hatchery and wild salmonids may have potentially damaging effects on wild populations (Reisenbichler and McIntyre 1977; Chilcote et al. 1986).

We used the model developed by Iwama and Tautz (1981) to facilitate comparison of growth among fish of different sizes being reared in two different environments. The model contains an exponent ($W^{0.333}$) to compensate for differences in weight and a linear adjustment for temperature differences. In this study, the model more successfully explained the growth of domesticated rainbow trout than the more seasonally variable performance of the steelhead trout. Thus, the model was sensitive to genotypic influences. This confirms the need for caution indicated by Wangila and Dick (1988) in the use of universal constants in comparative studies on fish growth. Jobling (1983) also proposed the use of an exponent to adjust for the effects of weight on growth. More powerful models could incorporate the effects of life history variability, especially for the comparison of domesticated and wild populations. Nevertheless, if its limitations are kept in mind, the Iwama and Tautz model remains useful for comparative studies because it adjusts for the effects of size- and temperature-related constraints on growth.

The performance of the hybrid trout in this study was in almost all cases intermediate to that of the pure steelhead families and the pure rainbow trout family maintained as a control. This indicates that much of the genetic differentiation between the rainbow and steelhead populations sampled in this study was due to additive genetic effects. Although the single family of rainbow trout was inadequate to provide a measure of within-population variation, the performance of this family was consistent with prior observations on the growth and seawater adaptability of domesticated rainbow trout. In addition to differences in growth patterns, a series of 24-h seawater challenge tests (Blackburn and Clarke 1987) on the groups in this study revealed a higher amplitude of seasonal variation in hypoosmoregulatory capacity in steelhead trout compared with hybrid trout (McLeese et al., unpubl. data). These findings are in agreement with a previous study on two other populations of rainbow and steelhead trout (Johnsson and Clarke 1988). In that experiment, exposure to simulated spring daylength during winter accelerated the appearance of decreased CF, as well as increased silvering and seawater adaptability in steelhead trout but not in domesticated rainbow trout. Because seawater adaptation in steelhead trout is size dependent (Johnsson and Clarke 1988), the slower growth of the ESW steelhead could have been a consequence of smaller size at the time of transfer to seawater. However, this is unlikely because the ESW steelhead initially grew well in seawater and weighed 20% more than the LSW steelhead in March, 3 mo after seawater transfer of the ESW groups. Thereafter, growth rates of the ESW steelhead decreased. It is likely that premature transfer to seawater disrupted the parr–smolt transformation (Hoar 1976; Folmar and Dickhoff 1980) causing endocrine abnormalities and suppression of growth, as has been demonstrated previously in coho salmon (*Oncorhynchus kisutch*) (Clarke and Nagahama 1977; Nishioka et al. 1982).

Intraspecific genetic variation in the growth response to seasonal photoperiod cues is not limited to rainbow trout. The two life history types of chinook salmon (*O. tshawytscha*) have very different growth responses to photoperiod (Clarke et al. 1989, 1992). Hanke et al. (1989) measured a family by photoperiod interaction in Atlantic salmon (*Salmo salar*) due to a change in the magnitude of the growth response among families. The degree of genetic differentiation between the natural population of rainbow trout used to establish the domesticated population examined in this study and the wild steelhead populations sampled is not known. However, many of the growth characteristics exhibited by the domesticated trout are those expected as the result of releasing fish from adaptive constraints on fast growth, and likely arose as the result of artificial selection for fast growth in the domesticated population. Thus, selection in the domesticated trout population has led to a reduced dependence on environmental cues such as temperature and photoperiod in the regulation of foraging activity and the ability to osmoregulate in seawater. Hybridization of wild steelhead trout with the domesticated rainbow trout produced the expected reduction in seasonality of growth performance in the hybrids compared with pure steelhead trout.

Acknowledgements

Excellent technical assistance was provided by John Blackburn, Bill Damon, John Shelbourn, and Alvin Solmie. John Morgan and Harry Orenius gave additional help with egg and milt collection and transportation. Jörgen Johnsson was supported by the Swedish Institute, "Oscar Ekmans Understödsfond," the Swedish Council for Forestry and Agricultural Research, and "Stiftelsen Västerhavet."

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Breakdown and Invertebrate Colonization of Leaf Litter in Two Contrasting Streams: Significance of Oligochaetes in a Large River

E. Chauvet

Centre d'Ecologie des Ressources Renouvelables, C.N.R.S., 29, rue Jeanne Marvig 31055 Toulouse Cédex, France

N. Giani

Laboratoire d'Hydrobiologie, Université Paul Sabatier, 118, route de Narbonne, 31062 Toulouse Cédex, France

and M. O. Gessner[1]

Centre d'Ecologie des Ressources Renouvelables, C.N.R.S., 29, rue Jeanne Marvig, 31055 Toulouse Cédex, France

Chauvet, E., N. Giani, and M. O. Gessner. 1993. Breakdown and invertebrate colonization of leaf litter in two contrasting streams: significance of oligochaetes in a large river. Can. J. Fish. Aquat. Sci. 50: 488–495.

The breakdown of willow (*Salix alba*) and beech (*Fagus silvatica*) leaves and their colonization by invertebrates were studied in a third-order mountain stream (breakdown rate $k = 0.0147$ and $0.0052 \cdot d^{-1}$, respectively) and a seventh-order lowland river ($k = 0.0094$ and $0.0027 \cdot d^{-1}$). Willow leaves broke down three times faster than beech leaves; this difference was twice that between streams. Total invertebrate densities and biomass and the proportion of shredders were higher in the large river; therefore, differences in breakdown capacities between streams cannot be attributed to differences in invertebrate community structure. Instead, microbial processing and abiotic fragmentation are suggested as factors controlling breakdown rates. Owing to the abundance of naidids, up to 2000 invertebrates per leaf bag were recorded in the large river. These oligochaetes appeared to use litter accumulations as a microhabitat that provided shelter and a rich supply of food in the form of fine particulate organic matter. Tubificid oligochaetes accounted for 55% of total biomass on partly degraded leaves at this site. Their abundance and regular occurrence inside willow leaves suggest that tubificids enhance leaf fragmentation and possibly mediate the incorporation of organic matter in river sediments once the plant tissue is sufficiently macerated.

La décomposition de feuilles de saule (*Salix alba*) et de hêtre (*Fagus silvatica*) et leur colonisation par les invertébrés ont été étudiées dans un torrent d'ordre 3 (taux de décomposition respectifs $k = 0{,}0147$ et $0{,}0052 \cdot d^{-1}$) et dans un fleuve d'ordre 7 ($k = 0{,}0094$ et $0{,}0027 \cdot d^{-1}$). Les feuilles de saule étaient dégradées trois fois plus vite que celles de hêtre et cette différence était deux fois plus importante que la différence entre rivières. La densité et la biomasse totales d'invertébrés ainsi que la proportion de dilacérateurs étaient plus élevées dans le fleuve; les différences de taux de décomposition entre les deux rivières ne pouvaient donc pas être attribuées à des différences dans la structure des communautés d'invertébrés, mais plutôt à la fragmentation abiotique et à l'activité microbienne. Les sachets de litière récoltés dans le fleuve contenaient jusqu'à 2 000 invertébrés, représentés essentiellement par des Naididés. Ces oligochètes utilisent les accumulations de feuilles comme un microhabitat leur procurant un abri et une source de nourriture sous la forme de fines particules de matière organique. Dans le fleuve, les oligochètes Tubificidés représentaient 55 % de la biomasse totale sur les feuilles partiellement décomposées. L'abondance et la présence régulière des Tubificidés à l'intérieur des feuilles suggèrent leur activité dans la fragmentation du matériel végétal macéré et dans l'incorporation de matière organique dans le sédiment.

Received March 31, 1992
Accepted September 14, 1992
(JB456)

Reçu le 31 mars 1992
Accepté le 14 septembre 1992

The importance of allochthonous carbon supplies for the metabolism of running waters is central to current stream ecosystem theory as formalized in the River Continuum Concept (Minshall et al. 1985; Statzner and Higler 1985). While this concept is operative at the scale of the drainage network, i.e. from small headwaters to major rivers, studies on the breakdown of terrestrial litter in streams are largely confined to low-order systems. A review of more than 100 studies revealed that 85% of them were conducted in streams of order 3 or lower and less than 4% were carried out in major rivers (stream order ≥ 7, e.g. Mathews and Kowalczewski 1969; Benfield et al. 1979; Chauvet 1988; Chergui and Pattee 1988). Apparently, leaf breakdown has not been considered an important process in these systems because increasing channel width results both in reduced shading, allowing enhanced primary productivity, and in decreasing amounts of allochthonous organic matter being imported per unit surface area, resulting in a gradual shift from heterotrophy to autotrophy in a downstream direction (Minshall et al. 1983, 1985).

Although less important than in headwaters, direct, airborne litter inputs to large rivers can be significant (Mathews and Kowalczewski 1969; Conners and Naiman 1984). Chauvet and Jean-Louis (1988), for example, estimated the annual supply of coarse particulate organic matter (CPOM) to a seventh-order stretch of the River Garonne to be 6 $t \cdot km$ stream length^{-1}, equivalent to 42 $g \cdot m^{-2}$ for the studied stretch. This represents nearly 10% of the average input per square metre to small

[1]Current address: Projektzentrum Ökosystemforschung, Christian-Albrechts-Universität, Schauenburger Str. 112, 2300 Kiel 1, Germany.

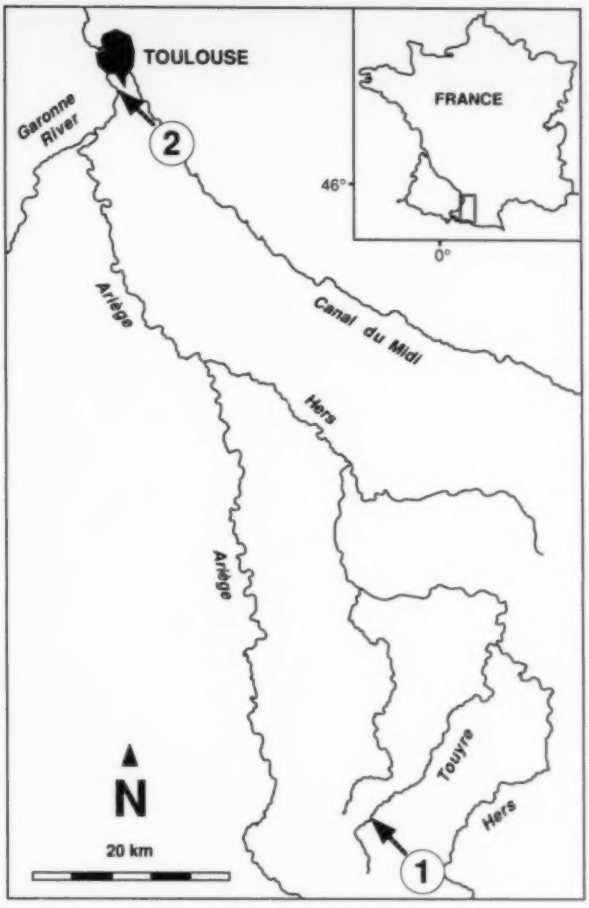

FIG. 1. Location of the Touyre and the Garonne River in southwestern France. Numbers refer to the study sites chosen along these streams.

streams with fully closed canopies (for references, see Webster et al. 1990). In addition to these direct inputs, there may be lateral imports of tree leaves from the floodplain (Conners and Naiman 1984; Shure and Gottschalk 1985), which may sometimes greatly exceed the total inputs normally measured for small streams (Cuffney 1988).

Conceptually, the aquatic invertebrate community changes along the upstream–downstream continuum from one codominated by large particle detritivores (shredders) and fine particle detritivores (collectors) to one predominantly composed of collectors, with scrapers assuming significant importance only in middle reaches (Minshall et al. 1983; Cummins 1988). Microbial assemblages may undergo similar changes, with leaf-degrading microfungi (aquatic hyphomycetes), for example, being more abundant in upstream reaches, although to our knowledge, there is no direct evidence supporting this idea. The patterns of litter breakdown should be affected by such changes in community structure, since the breakdown of detritus is a community process (sensu Reice 1974) with both microorganisms and invertebrates being involved. This hypothesis ties in with the observation that invertebrates appear to be critical for organic matter degradation in many low-order streams (Webster and Benfield 1986; Cuffney et al. 1990) but tend to be of minor importance in high-order rivers (Mathews and Kowalczewski 1969; Benfield et al. 1979; Chergui and Pattee 1988).

TABLE 1. Physical parameters and water chemistry of the two sites. For the discharge, temperature, and chemical data, minimum and maximum values during the experimental period are given. Discharge of both streams peaks in spring during snowmelt.

Parameter	Touyre	Garonne River
Stream order	3	7
Distance from source (km)	9	193
Catchment area (km^2)	25	9980
Altitude (m)	870	139
Mean width (m)	5	80
Mean depth (m)	0.5	2
Channel gradient (%)	5.5	0.1
Substrate composition	Sand, rocks	Silt
Discharge ($m^3 \cdot s^{-1}$)	0.5–4.3	104–765
Temperature (°C)	0–2	4–7
Oxygen (% saturation)	>100	80–100
pH	6.0–6.8	7.6–8.0
Alkalinity ($meq \cdot L^{-1}$)	0.2–0.7	1.2–2.6
[$N\text{-}NO_3^-$] ($mg \cdot L^{-1}$)	0.1–0.3	0.4–1.3
[$P\text{-}PO_4^{3-}$] ($mg \cdot L^{-1}$)	0–0.02	0.01–0.15

In this study, we compare the breakdown of leaf litter in two contrasting running waters, a third-order mountain stream and a seventh-order lowland river, both situated within the same drainage basin. Our objective was twofold: first to determine the importance of location in the drainage basin for controlling rates of breakdown relative to the prevailing "leaf-species effect" (Petersen and Cummins 1974; Webster and Benfield 1986) and second to evaluate the assemblages of invertebrates associated with leaf packs. Special emphasis was placed on oligochaetes because these invertebrates may be an important component of the leaf pack associated fauna (Mayack et al. 1989) but are not normally considered in studies of leaf litter breakdown in freshwater ecosystems.

Sites, Materials, and Methods

The study was conducted in two streams of the Garonne River basin in southwestern France (Fig. 1): the Touyre, a third-order stream in the French Pyrenees (site 1), and the Garonne River itself which, at the study site, is a seventh-order lowland river (site 2). Morphometric, physical, and chemical characteristics of the streams are given in Table 1. The study site chosen on the Touyre was located some 1000 m downstream from the site described previously (Gessner 1991). Here, the valley is narrow, resulting in the suppression of a typical riparian vegetation, and therefore, major inputs consist of litter from the upland forest, mainly in the form of beech leaves (*Fagus silvatica* L.). The station on the Garonne River corresponds to the "slow flowing water" site of Chauvet (1988) where the river is lined by nearly monospecific stands of willow (*Salix alba* L.).

To study leaf breakdown and the colonization of leaf packs by invertebrates, a crosswise experiment was designed with beech and willow leaves, i.e. the species indigenous to the Touyre and to the Garonne River, respectively, being incubated in both streams. Leaves were collected at abscission, dried for 3 d at 50°C, and 4-g portions placed in nylon mesh bags with 4-mm mesh openings. The bags were attached to concrete blocks and placed in the streams on 16 December 1986. Three replicate samples of each species were removed after 4, 8, 12, and 16 wk; additional samples were taken in the Garonne River after 20 and 24 wk.

TABLE 2. Exponential breakdown rates (d^{-1}) ± 95% asymptotic confidence limits.

Site	Beech (d^{-1})	Willow (d^{-1})	Willow:beech[a]
Touyre	0.0052 ± 0.0021	0.0147 ± 0.0029	2.83
Garonne River	0.0027 ± 0.0003	0.0094 ± 0.0010	3.48
Touyre:Garonne[a]	1.93	1.56	

[a]Ratio of breakdown rates.

TABLE 3. Initial composition of beech and willow leaf litter (% of dry mass ± 95% confidence limits).

Constituant	Beech	Willow
Carbon	49.1 ± 0.7	41.7 ± 0.3
Nitrogen	1.08 ± 0.02	1.64 ± 0.08
Lignin	29.9 ± 0.2	20.0 ± 1.2

In the laboratory, the leaves were rinsed in tap water, and then all animals were sorted under a dissecting microscope and preserved in 4% formalin. The invertebrates from the three replicate bags were pooled. They were later identified, mostly to species or genus, dried at 105°C for 1 d, and weighed to the nearest 0.1 mg. Numbers of very small and numerous organisms (mainly naidids from the Garonne River samples) were generally estimated by subsampling the pooled samples. Invertebrates were assigned to functional feeding groups according to various literature sources (e.g. Merritt and Cummins 1984; Iversen 1988). *Gammarus* and *Asellus* were placed in the shredder feeding group, although at least small animals of these taxa are known to feed on fine particulate organic matter (FPOM) (e.g. Gee 1988).

The leaf material was dried for 3 d at 50°C, weighed, and ground to pass a 1-mm-mesh screen. Organic matter content was measured as mass loss on ignition (2 h at 550°C); the initial contents of carbon, nitrogen, and lignin were determined according to standard procedures described by Chauvet (1987, 1988). The losses in ash-free dry mass (AFDM) of leaves through time were fitted to an exponential model of the $m_t = m_0 e^{-kt}$ type (Boulton and Boon 1991) where m_t represents the mass remaining at time t, m_0 the initial mass, and k the breakdown rate constant. The parameters were estimated by nonlinear regression analysis using the SAS statistical package.

Results

After 4 mo of decomposition, 21% of willow and 47% of beech AFDM remained in the Touyre, while as much as 42 and 74% of the willow and beech leaves incubated in the Garonne River were still left after nearly 6 mo. Both willow and beech thus decomposed at considerably slower rates in the large lowland river (Table 2). Within both streams, willow decomposed significantly faster than beech, and the comparison of the ratios of breakdown coefficients revealed that this "leaf–species effect" was roughly twice as large as the "stream effect" (Table 2). The faster breakdown of willow reflected the higher nitrogen and lower lignin content (Table 3) and thus the higher quality of this litter type for the leaf-consuming biota.

The leaf-associated fauna differed markedly between streams, but within either stream, differences between leaf types were small (Table 4). A total of 71 and 82 invertebrate taxa were recorded in the Touyre and the Garonne River, respectively, and oligochaetes accounted for about half of this richness (Appendix). Note, however, that other invertebrate groups such as chironomids were not identified to the same taxonomic level as oligochaetes.

Although numbers of invertebrates per leaf bag fluctuated considerably through time (Fig. 2), invertebrate densities were consistently found to be much higher on leaf litter incubated in the Garonne River. This was mainly due to the extremely high numbers of oligochaetes, with peak values reaching some 800 organisms·g willow leaves^{-1} and more than 2000·g beech leaves^{-1}, many of which were naidids, especially during the first 3 mo of decomposition. Animal densities in the Touyre, by contrast, never exceeded 100 animals·g leaf material^{-1}.

When expressed in terms of biomass, differences between the two streams remained, although they were less prominent (Fig. 3). Oligochaetes were still dominant in the Garonne River, but the naidid component declined in relative importance, while the proportion of the shredding crustaceans *Gammarus* cf. *pulex* L. and *Asellus aquaticus* L. and of chironomid collectors increased (Table 4). Percentages of the functional feeding groups tended to differ between streams, while differences related to leaf type were comparatively low (Table 5). Shredders were the dominant group in the Garonne River, with an average of almost 50% of total invertebrate biomass, but they represented only about 25% in the Touyre. In this stream predatory animals accounted for a great portion of total invertebrate biomass, often more than 30%, whereas in the large river predators represented less than 9% on average. Collector–gatherers made up nearly half of the invertebrate biomass in leaf bags of the Garonne River, with naidid oligochaetes, tubificid oligochaetes, and other collectors (mainly chironomids) being equally important when all samples were considered together. The changing pattern through time was strongly influenced by the dynamics of Tubificidae (Fig. 4): after 12–16 wk, their biomass had increased to 54–56% of the total invertebrate biomass, the maximum being reached later on beech than on willow, and then the biomass decreased toward the end of the sampling period. Grazers were not found to be associated with leaf bags in either of the two streams.

Discussion

Shredders, Predators, and Leaf Breakdown

The position of the breakdown site in the drainage network significantly influenced leaf breakdown rates, with faster breakdown occurring in the headwater for both leaf species studied (Table 2; analysis of covariance on log-transformed data, $P < 0.05$). This is in accordance with both theoretical predictions (Minshall et al. 1983) and experimental data on leaf breakdown in large rivers (e.g. Mathews and Kowalczewski 1969; Benfield et al. 1979; Chauvet 1987; Chergui and Pattee 1988), although a general upstream–downstream trend could not be conclusively demonstrated in the one study attempting to measure breakdown rates along the river continuum (Minshall et al. 1983).

The reduced breakdown rates in the Garonne River cannot be attributed to classical parameters such as temperature, pH, or nutrient concentrations of the stream water because all these factors were more favorable (see Webster and Benfield 1986) at the seventh-order site (Table 1). Oxygen concentrations in the water column of the Garonne River were also sufficient to permit a high biological activity (Table 1), although critically low levels may still have occurred at the sediment–water interface where leaf bags were exposed. However, blackened

TABLE 4. Biomass (mg) of invertebrates associated with each set of three leaf bags. Only taxa with biomass higher than 0.1 mg are figured. The functional feeding groups (FFG) are indicated as shr = shredders, col = collector–gatherers, fil = collector–filterers, and pre = predators, ADFM = ash-free dry mass remaining.

		Incubation time of beech leaves (wk)						Incubation time of willow leaves (wk)					
Taxon	FFG	4	8	12	16	20	24	4	8	12	16	20	24
Touyre													
Oligochaeta													
Eiseniella	col	5.0	20.7	14.0	—			63.3	17.0	4.6	36.3		
Ephemeroptera													
Habroleptoides	col	—	—	—	—			—	0.6	—	—		
Plecoptera													
Taeniopteryx	shr	4.1	—	—	—			—	—	—	—		
Protonemura	shr	1.4	—	—	5.1			—	1.0	—	—		
Amphinemura	shr	1.0	2.3	—	1.4			4.2	5.5	1.2	—		
Nemoura	shr	2.2	1.7	—	—			1.1	1.3	—	—		
Leuctra	shr	7.5	5.2	—	—			2.3	2.8	—	—		
Isoperla	pre	3.2	—	—	2.0			0.8	—	—	—		
Siphonoperla	pre	9.0	1.1	—	—			—	—	—	—		
Trichoptera													
Hydropsyche	fil	1.4	—	1.7	25.8			—	0.8	—	—		
Limnephilidae	shr	0.7	2.3	—	—			9.8	2.6	—	—		
Odontocerum	shr	—	—	—	27.6			—	—	—	—		
Micrasema	shr	—	4.6	2.4	6.1			—	1.6	—	—		
Sericostomatidae	shr	—	1.7	—	12.3			34.9	37.8	—	—		
Diptera													
Dicranota	pre	1.5	1.6	—	—			—	—	—	—		
Psychodidae	col	—	0.7	—	—			—	—	—	—		
Simuliidae	fil	—	—	1.5	14.1			—	—	1.0	—		
Athericidae	pre	6.0	42.5	9.9	20.9			55.1	38.9	—	—		
Tanypodinae	pre	—	1.5	—	—			—	2.6	—	—		
Orthocladiinae	col	—	1.0	—	2.4			—	—	—	—		
Total		43.0	86.9	29.5	117.7			171.5	112.5	6.8	36.3		
Leaf AFDM (g)		9.6	8.7	7.1	4.7			5.7	3.5	1.8	0.2		
Garonne River													
Hydrozoa													
Hydra	pre	—	—	1.0	—	12.5	—	—	—	11.4	0.5	1.2	—
Oligochaeta													
Naididae	col[a]	—	56.8	185.2	—	30.0	—	—	3.2	54.0	—	29.1	21.5
Tubificidae	col	—	—	15.0	13.5	175.6	21.3	—	5.0	27.6	61.8	15.3	26.3
Amphipoda													
Gammarus	shr	7.0	11.8	172.4	30.0	10.0	3.1	1.6	98.6	—	11.3	17.3	25.8
Isopoda													
Asellus	shr	6.9	18.1	95.2	94.0	27.3	94.1	24.9	36.6	24.3	4.5	9.3	175.5
Ephemeroptera													
Ephemera	col	—	—	—	—	—	—	2.0	—	—	2.0	—	—
Potamanthus	col	1.4	—	—	2.1	1.9	—	—	—	0.8	10.8	—	—
Caenis	col	—	0.9	—	1.4	5.0	4.5	—	1.2	—	—	1.7	2.8
Odonata	pre	—	—	5.6	5.1	5.3	—	13.3	26.1	—	—	—	—
Trichoptera													
Polycentropus	pre	—	1.7	5.0	1.9	1.9	—	0.9	—	9.7	—	—	—
Cyrnus	col	—	5.3	5.9	6.8	5.7	4.0	1.6	1.2	9.5	3.1	0.7	—
Hydropsyche	fil	—	0.3	—	1.7	—	—	—	—	—	—	—	—
Diptera													
Athericidae	pre	—	—	2.5	—	—	—	—	—	2.0	—	—	—
Tanypodinae	pre	—	13.5	—	4.4	17.0	1.0	—	—	7.2	2.7	—	—
Orthocladiinae	col	—	16.0	127.6	7.4	—	—	—	10.0	18.2	2.9	—	12.0
Chironomini	col	—	—	—	2.9	19.3	23.3	1.4	6.0	2.1	11.7	4.3	19.5
Tanytarsini	col	—	0.7	22.0	4.4	—	—	0.4	0.6	16.2	2.9	3.3	0.7
Total		15.3	125.1	637.4	175.6	311.5	151.3	46.1	188.5	183.0	114.2	82.2	284.1
Leaf AFDM (g)		8.9	8.3	7.7	4.9	7.1	6.8	5.9	5.1	4.4	3.9	2.9	2.1

[a]Also some grazers and a few predators.

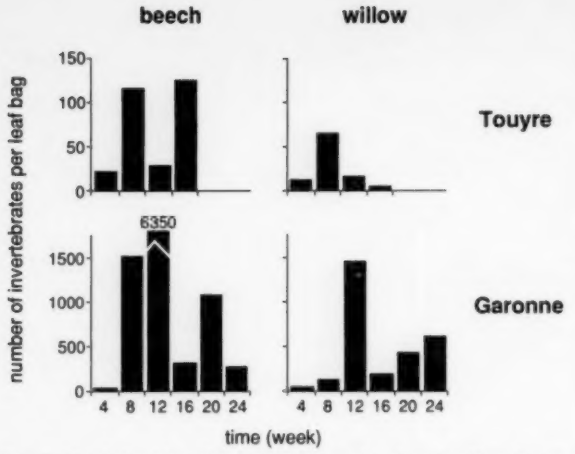

FIG. 2. Total densities of invertebrates on beech and willow leaves during breakdown in the Touyre and the Garonne River.

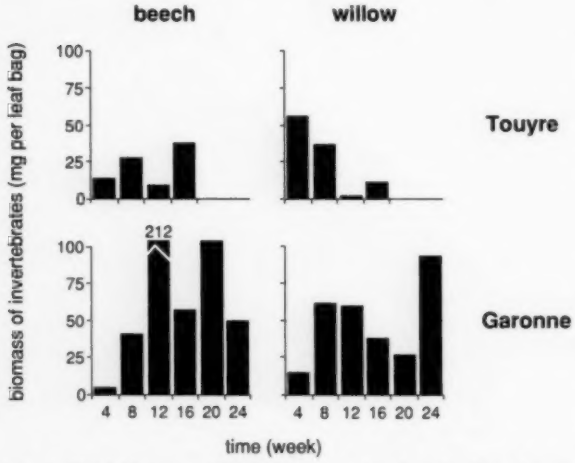

FIG. 3. Total biomass of invertebrates on beech and willow leaves during breakdown in the Touyre and the Garonne River.

leaves or an odor of H_2S that would have indicated anoxic conditions were never observed.

According to the River Continuum Concept, we initially hypothesized that shredders would be more abundant in leaf bags submerged in the low-order stream, leading to faster breakdown of leaf litter in this sytem. In contrast with this prediction, however, members of this functional feeding group (essentially *G.* cf. *pulex* during the first months of decomposition and *A. aquaticus* toward the end of the study period) reached a much higher biomass in the leaf bags submerged in the Garonne River (Table 5). Although numbers and biomass do not necessarily indicate feeding rates, it is clear that differences in shredder abundance cannot explain the observed differences in breakdown rates.

In the Touyre, shredders are normally dominated by filipalp stoneflies, especially of the genus *Protonemura* (Gessner and Dobson 1993). In the present study, however, these taxa were only rarely encountered in leaf bags, and this probably explains the relatively low proportion of shredders, which represented only about one quarter of the total invertebrate biomass (Table 5). Concurrently, the piercer *Atherix* cf. *ibis* F. and other carnivores made up an exceptionally large fraction of biomass, suggesting significant feeding pressure on the shredder community. This phenomenon has previously been reported for macroinvertebrates associated with maple leaf litter in a Rocky Mountain stream (Oberndorfer et al. 1984), but in contrast with the conclusions drawn by those authors, shredder predation did not lead to a drastic reduction of breakdown rates in the present investigation.

Given the greater abundance of invertebrates in the Garonne River and the larger proportion of shredders, we are tempted to attribute the slower breakdown in this high-order system to differences in the composition, abundance, and activity of the microbial communities associated with the leaf litter incubated in the two study streams. These differences would probably be related to siltation, the composition of river sediments (Table 1), or both, resulting in a reduced availability of oxygen and the creation of a physically unfavorable environment for the leaf-associated microbiota. In the Touyre, the biomass of leaf-degrading fungi (aquatic hyphomycetes) associated with leaf litter can be very high, with peak values reaching 9% of total detrital mass (Gessner and Schwoerbel 1991). Unfortunately, comparable data are not currently available for the Garonne River, but reduced sporulation rates of these fungi (Chauvet et al. 1986 and unpubl. data) point to lower fungal activity in this system. If not counterbalanced by an increased activity of bacteria, this would result in lower breakdown rates.

An additional or an alternative cause of faster breakdown in the Touyre may have been an increased loss of particles, resulting from greater mechanical breakage. The high gradient of this stream coupled with the roughness of the streambed (Table 1) leads to high turbulence and thus elevated shear stress acting upon the leaf packs, especially during periods of spates following snowmelt. As judged from the physical appearance of leaves upon recovery, this mechanism was unlikely, however, to account entirely for the faster breakdown in the Touyre, except for beech during later stages of breakdown.

Cummins et al. (1989) suggested that leaf litter dynamics can be modelled on a catchment basis when information on the magnitude, timing, and quality of litter input, instream retention capacities, and the breakdown coefficient of different litter types (leaf species or breakdown categories) is known. Clearly, the position of the breakdown site along the river continuum is an additional variable to be taken into account. In spite of different absolute breakdown coefficients, the ratios of breakdown rates for the two leaf species were roughly equal in the two streams (Table 2). Similarly, the ratio of breakdown rates in the two streams did not vary much from one species to the other. Provided, therefore, that the relative constancy of these ratios is real and not simply a random outcome of our limited study with two leaf species at two sites, projections of leaf litter turnover could be made for a given drainage system with reasonable labor investment by measuring breakdown rates of n leaf species (or breakdown categories) at one site and of one reference species at m representative sites within the drainage network.

Naidid Oligochaetes

Numbers of invertebrates associated with leaf litter in the Garonne River greatly exceeded maxima observed in other breakdown studies. This was obviously due to the abundance of small organisms, particularly naidid oligochaetes. These invertebrates are not usually considered in comparable inves-

TABLE 5. Percentage of the biomass of the invertebrate feeding groups (FFG) during time and mean percentage during breakdown (% of total biomass).

FFG	Incubation time of beech leaves (wk)						Incubation time of willow leaves (wk)					
	4	8	12	16	20	24	4	8	12	16	20	24
Touyre												
Shredders	39	20	8	45			30	47	17	0		
Collector–gatherers	12	26	47	2			37	16	68	100		
Collector–filterers	3	0	11	34			0	1	15	0		
Predators	46	54	34	19			33	37	0	0		
Garonne River												
Shredders	91	24	42	71	12	64	57	72	13	14	32	71
Collector–gatherers	9	64	56	22	77	36	12	14	70	84	66	29
Collector–filterers	0	0	0	1	0	0	0	0	0	0	0	0
Predators	0	12	2	6	12	1	31	14	17	3	1	0

tigations, which tend to focus on the macrofaunal assemblage on leaf litter. Naidids represented up to 73% of the total number of organisms collected on willow leaves and 65% on beech leaves and occurred primarily during the first 3 mo of decomposition. These small, highly mobile animals feed on a variety of food items, but especially on FPOM including bacteria and unicellular algae such as diatoms (Learner et al. 1978). Their feeding mode does not, however, allow shredding of CPOM such as leaf litter. Why, then, were these organisms so abundant in the leaf bags submerged in the Garonne River? Leaf accumulations provide large surface areas, promoting a rich development of microorganisms, and hence abundant food for fine particle feeders such as most of the naidid species identified in the present study (Table 4; Appendix). Learner et al. (1978) proposed a similar explanation for the distribution of naidids on macrophytes, based on the observation that plant species with highly dissected growth forms, which permit the highest standing stocks of periphyton, also support the densest populations of naidid oligochaetes. Leaf accumulations, like macrophytes, may also act as traps of fine particles in transport, a process that would further increase the supply of food for invertebrates of the collector–gatherer guild. Moreover, dense clumps of macrophytes afford shelter against the water current to naidids (Harrod 1964), and this may also work with leaf packs. In the present study, the samples taken in March, 2 d after a major spate, revealed large accumulations of naidids on the leaves, suggesting that leaf packs may indeed constitute a refuge during periods of physical instability. Overall, then, naidids seemed to be attracted to leaf packs mainly because of a surface area effect. This conclusion is supported by two further observations, namely the colonization of strips of synthetic tissue simulating natural leaves, which were incubated at the study site (unpubl. data), and the greater abundance of naidids on beech leaves (Appendix; even when expressed on a per gram AFDM remaining basis) whose initial surface to dry mass ratio was more than twice as high as that of willow leaves.

Tubificid Oligochaetes

While oligochaetes of the family Tubificidae were totally absent in the low-order mountain stream, they represented more than 50% of the animal biomass on willow leaves submerged in the Garonne River after a period of 4 mo (Fig. 4). On beech leaves, the maximum was equally high but occurred 1 mo later, probably due to the greater toughness of this litter type. At this stage, willow leaves were substantially degraded, with the epidermis peeling off and the tissue being fragmented into several

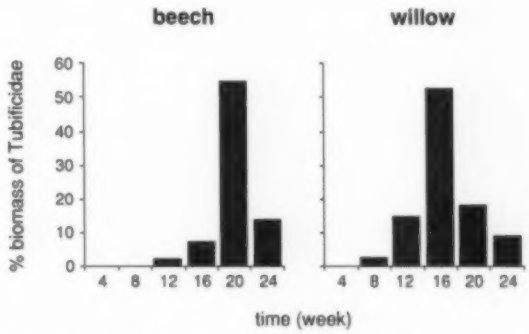

FIG. 4. Proportion of the oligochaetes Tubificidae on beech and willow leaves during breakdown in the Garonne River (% of the total invertebrate biomass).

layers. Tubificids were regularly encountered not only on the surface but also inside the leaf matrix, suggesting that the animals were feeding on saprotrophic bacteria proliferating in the foliar tissue and on the parenchymal cells themselves, liberated by the macerating activity of microorganisms such as aquatic hyphomycetes (Suberkropp and Klug 1980). In other words, microbial conditioning would have facilitated access of these soft-body organisms to the interior of leaves and hence tapping of a rich carbon and nutrient resource. Once inside the tissue, the worms could in turn enhance leaf breakdown by their moving about and their feeding activity. If migration between leaf accumulations and the sediments takes place, this would also lead to a biologically mediated incorporation of organic matter into the river sediments. We found some support for this hypothesis from a preliminary feeding experiment where conditioned leaf material lost mass more rapidly when tubificids were present. However, these results were poorly reproducible, so more careful experiments are needed in order to provide unequivocal answers to the issue.

Acknowledgments

We thank Michèle Escautier for technical assistance and Mike Dobson for constructive criticism of the manuscript.

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Appendix

Oligochaete taxa collected from leaf bags in the Garonne River. Numbers refer to individuals from pooled samples.

Taxon	Incubation time of willow leaves (wk)						Incubation time of beech leaves (wk)					
	4	8	12	16	20	24	4	8	12	16	20	24
Lumbriculidae												
Lumbriculus variegatus (Müll.)	—	—	—	—	2	2	—	—	—	—	—	—
Stylodrilus heringianus Clap.	—	—	22	—	—	—	—	—	—	—	—	—
Stylodrilus lemani (Grube)	—	—	—	1	—	—	—	—	—	—	—	—
Stylodrilus sp.	—	1	—	4	22	1	—	—	6	—	40	5
Naididae												
Chaetogaster diaphanus (Gruith.)	8	150	440	—	—	1	8	22	138	—	—	25
Chaetogaster diastrophus (Gruith.)	—	283	440	3	20	—	—	11	42	—	3	—
Amphichaeta leydigii Tauber	—	62	88	—	—	—	—	2	12	—	—	—
Paranais frici Hrabe	—	8	110	5	20	1	—	1	36	—	3	65
Specaria josinae (Vejd.)	2	61	198	11	81	4	2	1	6	1	43	55
Uncinais uncinata (Ørst.)	2	—	44	9	1	19	—	2	6	—	7	90
Ophidonais serpentina (Müll.)	3	39	132	17	64	11	3	1	132	13	40	65
Nais alpina Sper.	—	—	22	—	—	—	1	—	—	—	—	—
Nais bretsheri Mich.	17	911	4290	—	60	11	38	55	1092	2	17	107
Nais communis Pig.	8	582	3564	1	164	2	8	12	618	4	40	56
Nais elinguis Müll.	6	184	594	—	—	—	—	—	108	—	—	39
Nais pardalis Piguet	17	703	1584	—	—	—	11	35	498	1	10	—
Nais pseudobtusa Piguet	1	115	—	—	—	—	1	1	—	—	3	—
Nais variabilis Piguet	—	—	—	1	80	—	—	—	—	—	27	—
Slavina appendiculata (Udek.)	—	—	—	—	—	1	—	—	—	—	—	—
Vejdovskyella comata (Vejd.)	—	—	—	117	—	—	—	—	—	—	—	—
Vejdovskyella intermedia (Bret.)	2	362	2860	137	60	—	—	41	282	—	27	20
Stylaria lacustris (L.)	40	316	1033	17	721	102	69	32	324	3	510	290
Pristina aequiseta f. foreli Bourne	—	13	—	2	—	—	—	—	—	—	—	—
Pristina longiseta Ehrbg.	—	18	—	—	—	—	—	1	6	—	—	—
Tubificidae												
Limnodrilus hoffmeisteri Clap.	—	5	—	2	22	6	—	—	24	12	20	16
Limnodrilus udekemanius Clap.	—	—	—	1	1	6	—	—	—	28	—	—
Limnodrilus sp.	—	—	—	—	—	—	—	—	48	—	—	—
Potamothrix hammoniensis (Mich.)	—	—	—	—	1	—	—	—	—	1	—	—
Potamothrix moldaviensis (Vejd. augm. Mraz.)	—	—	—	3	41	3	—	—	—	27	20	14
Ilyodrilus templetoni (South.)	—	—	—	1	—	—	—	—	—	3	—	—
Aulodrilus limnobius Bret.	—	—	—	—	—	—	—	—	—	—	3	10
Aulodrilus pluriseta (Piguet)	—	—	—	2	—	—	—	—	—	—	3	—
Bothrioneurum vejdovskyanum Stolc	—	—	110	—	—	20	—	—	—	—	—	—
Bothrioneurum sp.	—	—	—	7	5	—	—	—	12	28	—	—
Branchiura sowerbyi Bedd.	—	—	—	1	27	3	—	—	6	8	3	6
Unidentified Tubificidae with hair setae	—	5	—	2	1	4	—	—	—	15	10	1
Unidentified Tubificidae without hair setae	—	13	44	121	627	199	—	2	—	233	240	312
Enchytraeidae												
Cernosvitoviella atrata (Bret.)	—	—	66	2	1	—	—	1	12	—	—	—
Enchytraeus sp.	2	1	1	—	—	—	—	—	1	—	—	—
Henlea sp.	—	—	—	—	1	—	—	—	—	—	—	—
Marionina argentea (Mich.)	—	—	—	—	—	—	—	—	—	1	—	—
Marionina riparia Bret. augm. Cern.	—	—	22	—	24	—	—	—	—	—	—	—
Total	108	3832	15664	467	2046	396	141	220	3409	380	1069	1176
Total (numbers·g AFDM remaining^{-1})	12	462	2034	95	288	58	24	43	774	97	369	560

Winds and the Demic Structure of a Population of Yellow Perch (*Perca flavescens*)

S. K. Aalto

Department of Mathematics and Computing Science, St. Francis Xavier University, Antigonish, N.S. B2G 1C0, Canada

and G. E. Newsome

Department of Biology, St. Francis Xavier University, Antigonish, N.S. B2G 1C0, Canada

Aalto, S. K., and G. E. Newsome. 1993. Winds and the demic structure of a population of yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 50: 496–501.

Variations in year-class strengths of populations of fish are often attributed to environmental effects such as wind or temperature. Previously, we have presented evidence showing that the yellow perch (*Perca flavescens*) in a lake are composed of demes or subpopulations that vary independently. Here, we explore the relationship between winds from different directions and the independent variations of the demes of yellow perch in the study lake. We present a model that includes an instantaneous rate of mortality due to winds during the embryonic stage of yellow perch. The variables used to explain the observed egg-mass counts and thus the variations in the size of the demes are lagged egg-mass counts and winds, each of which varies yearly. Using wind and egg-mass census data, we were able to find statistically significant models that explain much of the variation (52–61%) in the independently varying demes (four of six investigated) of yellow perch in our study lake. We also present data that show that water temperatures vary in different ways at different sites in the same year, and in different ways at the same site in different years.

On attribue souvent l'importance des effectifs de poissons par classe d'âge à des effets du milieu comme le vent ou la température. Nous avons précédemment fait état de résultats de nos travaux montrant que la population de perchaudes (*Perca flavescens*) dans un lac est composée de dèmes ou sous-populations qui varient de manière indépendante. Dans cet article, nous explorons les rapports entre différentes directions prises par le vent et des variations indépendantes de dèmes chez la perchaude du lac d'étude. Nous présentons un modèle à taux instantané de mortalité attribuable aux vents durant le stade embryonnaire de la perchaude. Le dénombrement différé des masses d'oeufs et le vent, chacun de ces facteurs variant d'une année à l'autre, sont les variables utilisées pour expliquer le nombre de masses d'oeufs observées et, par conséquent, les variations de l'importance des dèmes. Au moyen des relevés des vents et des masses d'oeufs, nous avons pu trouver des modèles statistiquement significatifs pour expliquer la majeure partie des variations (52–61 %) observées chez les dèmes qui varient de manière indépendante (quatre des six dèmes étudiés) de la perchaude dans le lac qui a servi à nos travaux. Nous présentons aussi des données montrant que la température de l'eau varie de manière différente en des emplacements différents au cours de la même année, et de manière différente au même emplacement d'une année à l'autre.

Received March 9, 1992
Accepted September 14, 1992
(JB430)

Reçu le 9 mars 1992
Accepté le 14 septembre 1992

Aalto and Newsome (1989, 1990) presented evidence that strongly suggests that the population of yellow perch (*Perca flavescens*) in Lochaber Lake, Nova Scotia, Canada, is divided into a number of distinct subpopulations or demes that vary independently of one another. This evidence is partly based on egg-mass counts that have been conducted at a variety of sites in Lochaber Lake (Newsome and Aalto 1987) and manipulations of egg masses. Here, we investigate the effect of winds in explaining the independent variations among the distinct demes.

The effects of wind and temperature on the development and survival of young-of-the-year (YOY) fish are frequently mentioned (Volodin 1960; Hokanson and Kleiner 1974; Busch et al. 1975; Clady 1976; Nelson and Walburg 1977; Guma'a 1978; Craig et al. 1979; Murray and Beacham 1987; Peterman and Bradford 1987; Taggert and Leggett 1987; Taylor et al. 1987; Stickland et al. 1988; Wiegand et al. 1988; Johannessen and Tveite 1989; Treasurer 1989; Upcroft 1989). Some authors noted that temperature extremes or variations may lead to deformities or death in hatching larvae (Volodin 1960; Hokanson and Kleiner 1974; Murray and Beacham 1987; Strickland et al. 1988; Wiegand et al. 1988).

Embryos of yellow perch at different sites in Lochaber Lake may experience different thermal regimes due to the effects of winds. The relationship between wind and temperature in lakes depends on a number of factors including wind direction and velocity, the size and shape of the lake, the topography of the lake bottom, and the temperature structure of the lake, the presence of a thermocline, and internal seiches (Cole 1975; Horn et al. 1986; Lemmin and Mortimer 1986; Simons and Schertzer 1987). The temperature regimes experienced by embryos in egg masses deposited at a particular spawning site will also depend on the factors mentioned as well as the individual topography of the site; thus, temperature regimes may vary from site to site, and under various wind conditions. This fact and the possibility that variations in temperatures may lead to deformities or death of hatching larvae provide an explanation for the differential effects of various winds on the year-class strengths of

demes of perch spawning in Lochaber Lake.

The purpose of this study is to show that winds from certain directions affect the variation in abundance of perch demes defined by different spawning sites in Lochaber Lake. Using explanatory models, we can demonstrate that a significant portion of the annual variation in the abundance of the independent perch demes can be explained by the effects of winds from specific directions.

Materials and Methods

Egg-mass census data for a number of sites in Lochaber Lake have been collected annually since 1978 (Newsome and Aalto 1987). Table 1 gives the sum of the egg-mass counts at the six sites (labelled 1, 2, 4, 6, 8, and 10) used in the analysis. These sites were chosen because they represented spawning locations utilized by the greatest number of females each year and because the data records are more complete. The locations of these sites are given in (Newsome and Aalto 1987). Lochaber yellow perch begin spawning in early June; peak spawning occurs during June 14–21 (Newsome and Aalto 1987). Our experiments suggest that Lochaber perch embryos hatch within 10–15 d.

Raw wind data from the observatory at the Halifax Airport were obtained from the Atmospheric Services Office, Environment Canada, Bedford, N.S., for the month of June for the years 1978–86. The data were organized so that for each hour of each day in June (which includes the spawning and embryonic periods for perch in Lochaber Lake) the hourly average speed (kilometres per hour) and direction of the wind were given. Wind directional information was encoded by dividing the compass into 16 equal (22.5°) directional sectors. Direction code 1 corresponds to northerly winds between $0 \pm 11.25°$; moving counterclockwise, subsequent 22.5° sectors are labelled one larger than the previous sector such that direction code 16 corresponds to the sector between 11.25 and 33.75°.

These data were subsequently processed to produce a number of wind indices for each direction and for two time periods: the month of June and the last 3 wk of June. Two classes of indices were computed: (1) the total number of hours during June when the wind velocity from a specific direction exceeded a specified minimum speed for 2 or more consecutive hours and (2) the sum of the velocities during those hours satisfying the conditions in (1). The minimum speeds selected were 10 and 15 km/h because whitecaps indicating the movement of water were observed to form in Lochaber Lake at these wind speeds. The requirement of 2 or more consecutive hours was selected to ensure that the effect was due to winds from a particular direction rather than combinations of directions.

For each direction, wind indices were computed for the years 1978–86. Wind indices were computed for the 16 directions and the sum of speeds for the minimum speeds of 10 and 15 km/h for June (labelled 10S and 15S), the last 3 wk of June (labelled 10S3W and 15S3W), and the number of hours (labelled 10H and 15H) for the selected minimum speeds. As expected, the wind indices show that winds from different directions are dominant in different years.

The number of egg masses deposited at a spawning site is equal to the number of mature female yellow perch utilizing that site each year. The number of egg masses counted at a given site is proportional to the number deposited. The following models relating the egg-mass counts c_t for various years t and an instantaneous rate of mortality BW_t due to the wind index W_t can be developed using the Leslie matrix model for a population of female yellow perch. If the female yellow perch population dynamics are largely explained by the dynamics of the age 3 age-class, then the following three-year model would explain the variations in the observed egg-mass counts (see Appendix):

$$c_{t+3} = Ae^{-BW_t}c_t + E.$$

If the population dynamics of the age 3 and 4 age-classes explain a large portion of the dynamics of the population, then the following four-year model would explain the variations in the observed egg-mass counts (see Appendix):

$$c_{t+3} = Ae^{BW_t}c_t + De^{-BW_{t-1}}c_{t-1} + E$$

The parameters A, B, D, and E in these models will be positive.

In Newsome and Aalto's (1987) study, age 3 female perch constituted 39% of all of the samples of mature female Lochaber yellow perch collected during 1978–82 and 1984–85, and age 3 and 4 females constituted 68% of all samples collected since 1978. Thus, a significant fraction of the egg masses deposited at all spawning sites was deposited by age 3 (and age 4) yellow perch.

Least squares fits of the three-year model to the various wind indices and egg-mass counts were computed. The technique used to determine A, B, and E is essentially the one presented by Allen (1966). The direction and wind index providing the smallest sum of squares error (SSE) for each site provided candidates for further consideration.

For the models with the smallest SSEs, adjusted coefficients of determination (Neter et al. 1989) are computed in order to see what proportion of the variance is explained by the nonlinear models. Tests (Seber and Wild 1989) of the hypotheses that $(A,B,E) = (0,0,e)$ where e is equal to the mean of the c_{t+3}, and that $(A,B,E) = (a,0,e)$ where a and e are the least squares coefficients for the linear model

$$c_{t+3} = ac_t + e$$

were conducted for selected directions and wind indices. The first of these two tests indicates whether the best fitting models explain any more than the average of the counts, while the second test determines whether there is an effect due to the wind directions and indices providing the best models (i.e. when $B = 0$, the three-year model reduces to this linear model). For

TABLE 1. Sums of the egg masses counted along the 4-, 6-, 8-, 10-, and 12-m transects for Site 1 and the 6-, 8-, and 10-m transects for Sites 2, 4, 6, 8, and 10 in Lochaber Lake for the years 1978–89.

Site	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989
1	139	229	250	170	172	132	98	91	69	134	105	105
2	27	18	21	15	23	7	15	8	8	32	11	22
4	17	55	60	35	51	45	15	36	13	26	28	34
6	36	36	56	11	16	39	14	17	25	60	31	42
8	10	12	10	3	10	22	2	10	8	38	20	10
10	4	25	17	2	22	22	3	18	3	10	18	15

TABLE 2. SSEs and directions providing the minimum SSE for each index and site (see text).

Index	Site					
	1	2	4	6	8	10
10S	4780, 2	227, 4	467, 2	817, 4	358, 4	339, 2
10H	4710, 2	248, 4	528, 2	905, 5	327, 4	329, 4
15S	4520, 2	209, 4	383, 2	609, 4	358, 4	309, 10
15H	4230, 2	241, 4	390, 2	613, 4	358, 4	285, 10
10S3W	6280, 2	276, 4	524, 10	974, 4	358, 4	255, 2
15S3W	5890, 2	228, 4	548, 2	619, 4	358, 4	237, 2

those directions and wind indices having the smallest SSEs for the three-year model, a four-year model was investigated using a nonlinear regression package (Hintze 1990). Tests of hypotheses similar to those above regarding the parameters (A, B, D, and E) of the four-year model were performed.

We conjecture that winds from certain directions affect the thermal regime experienced by developing perch embryos each year at each site, resulting in the observed independence of population dynamics among sites annually (Newsome and Aalto 1987; Aalto and Newsome 1989, 1990). To study the variation in thermal regimes, temperatures were recorded every 15 min at a depth of 9 m at Sites 5 and 16 (Newsome and Aalto 1987) during the June 14–26 period of 1990 and 1991 which corresponds to the developmental period for perch embryos.

Results

For each of the sites, there is a direction and a wind index, such that the three-year model has the smallest SSE between the model predictions and the actual egg-mass counts and which also had positive values for A, B, and E. For most directions and wind indices, the best SSE models either had one of A or E negative or $B = 0$ (no wind effect for that wind direction and index) or both. This supports our hypothesis that only certain winds will have an important impact in determining the annual recruitment success at each spawning site.

Table 2 shows the SSEs and directions for the best fitting three-year models for each wind index at the sites investigated. Most sites show a consistency of direction over the various wind indices. Most of the exceptional sites involve a wind from an adjacent direction, or from the opposite direction (e.g. for Site 4 the directions 2 and 10 provide the smallest SSEs for the various indices, and these two directions are opposites). For half of the sites the index providing the smallest SSE is 15S. For Site 1 the index providing the smallest SSE is 15H, for site 8 the smallest SSE occurs for 10H, and for Site 10 the smallest SSE occurs for 15S3W.

The 10H and 15H indices are less variable than the 10S and 15S indices. This shows that the effects of winds may be independent of velocities exceeding the minimum velocity. This could occur if a site is more exposed to the wind. Site 1 is large and somewhat distant from the shore (in comparison with the other sites) and so is more exposed to winds. Similarly, Site 8 on the east side of the lake is more exposed to winds from the west. This may explain why these two sites appear to be more sensitive to winds from the west. For Site 10 the indication is that the winds during the first week in June are less important and that possibly these perch spawn slightly later than do the others.

The adjusted coefficients of determination and results of the other tests are given in Table 3 for the three-year models having the smallest SSEs and for selected four-year models. The adjusted coefficients of determination varied between 0.42 and 0.75. For all sites except 1 and 10, there is an index and direction such that both hypotheses $(A,B,E) = (0,0,e)$ and $(A,B,E) = (a,0,e)$ are rejected ($p < 0.10$).

It is expected that the three-year and four-year models should have similar parameters because the topography of each site is the same each year. In particular, the parameter B reflecting the impact of winds on embryonic survival should be the same for the three- and four-year models. Examination of Table 3 shows that at Sites 4, 6, and 8 where four-year models were found, the pairs of values of B are very similar.

Figure 1 shows the variation in temperatures at Sites 5 and 16 between June 14 and 26 1990 and 1991. This time period corresponds to the period of development for the majority of perch embryos deposited annually at sites in Lochaber Lake. These graphs show that the temperature varies differently at each of the two sites (in each of the years the variation about the mean or trend is larger at Site 5 than at Site 16) in the same year and that the temperature varies differently at the same site during different years (the variation about the trend at both sites in 1990 is greater than the variation about the mean in 1991).

Discussion

For all sites except 8, the wind direction providing the smallest SSE is a wind blowing away from the shore near the spawning site. Such winds may cause upwelling of colder water from beneath the thermocline (Simons and Schertzer 1987).

For Sites 2, 4, 6, and 8 the method of investigation produced a three-year model that clearly demonstrates that winds can explain a large part of the variation in the egg-mass counts (52–61% of the variance is explained) and can thus affect the survival of yellow perch from the embryonic stage to the third year-class. For these sites, the direction and the wind index providing the best fitting model vary. This supports the hypothesis that each wind affects each site differently and produces different yearly survival rates for the yellow perch embryos at each site.

The nature of the relationship between the winds and the survival of embryonic perch to 3 and 4 yr old is determined by the overlap of the time interval of the computed wind index with the life cycle of the yellow perch. As noted above, the spawning and embryonic periods occur in June. Yellow perch larvae become pelagic 0–5 d after hatching (Hokanson and Kleiner 1974). Most of the YOY would be pelagic between June 22 and the end of June. For most sites, the period of the wind index providing the smallest SSE is the month of June. For Site 10 the period of the wind index providing the smallest SSE is the last 3 wk of June.

The winds during the early part of June will determine the distribution of the temperatures at the various depths in Lochaber Lake. For example, light winds may not mix the warming surface water with cold subsurface water as thoroughly as heavy winds, and this may result in a sharp thermocline at a shallow depth separating relatively warm water from cold water. Because one of the cues for perch spawning behaviour is temperature, the winds may indirectly affect the depths at which Lochaber perch elect to spawn.

The distribution of temperatures just prior to spawning may also determine the subsequent thermal regime experienced by developing embryos. For example, if a sharp thermocline formed prior to spawning, then after the egg masses were deposited, certain winds could produce internal seiches that

TABLE 3. Coefficients, SSEs, adjusted coefficients of determination (ACD), and statistical significance for selected models of the form

$$c_{t+3} = Ae^{-BW_t}c_t + E$$

and

$$c_{t+3} = Ae^{-BW_t} + De^{BW_{t-1}}c_{t-1} + E.$$

p_1 indicates the significance of the first and p_2 the significance of the second null hypothesis (see text).

Site	Index	Direction	A	B	D	E	SSE	ACD	p_1	p_2
1	15H	2	0.56	0.082	—	85	4233	0.4309	0.14	0.20
2	15S	4	1.5	0.0051	—	10	209	0.5153	0.09	0.09
4	15S	2	1.4	0.0046	—	15	384	0.5934	0.05	0.08
	15H	2	1.3	0.086	—	15	390	0.5866	0.06	0.08
	15H	2	1.4	0.096	0.031	16	375	0.4718	0.22	0.30
6	15S	4	3.0	0.0079	—	18	609	0.6147	0.05	0.05
	15S	4	3.0	0.006	1.4	9.3	265	0.7476	0.06	0.06
8	10H	4	33	0.19	—	7.5	327	0.5738	0.06	0.09
	10H	4	33	0.19	13	5.3	164	0.6781	0.09	0.19
10	15S3W	2	1.1	0.0035	—	5.5	237	0.4184	0.15	0.37

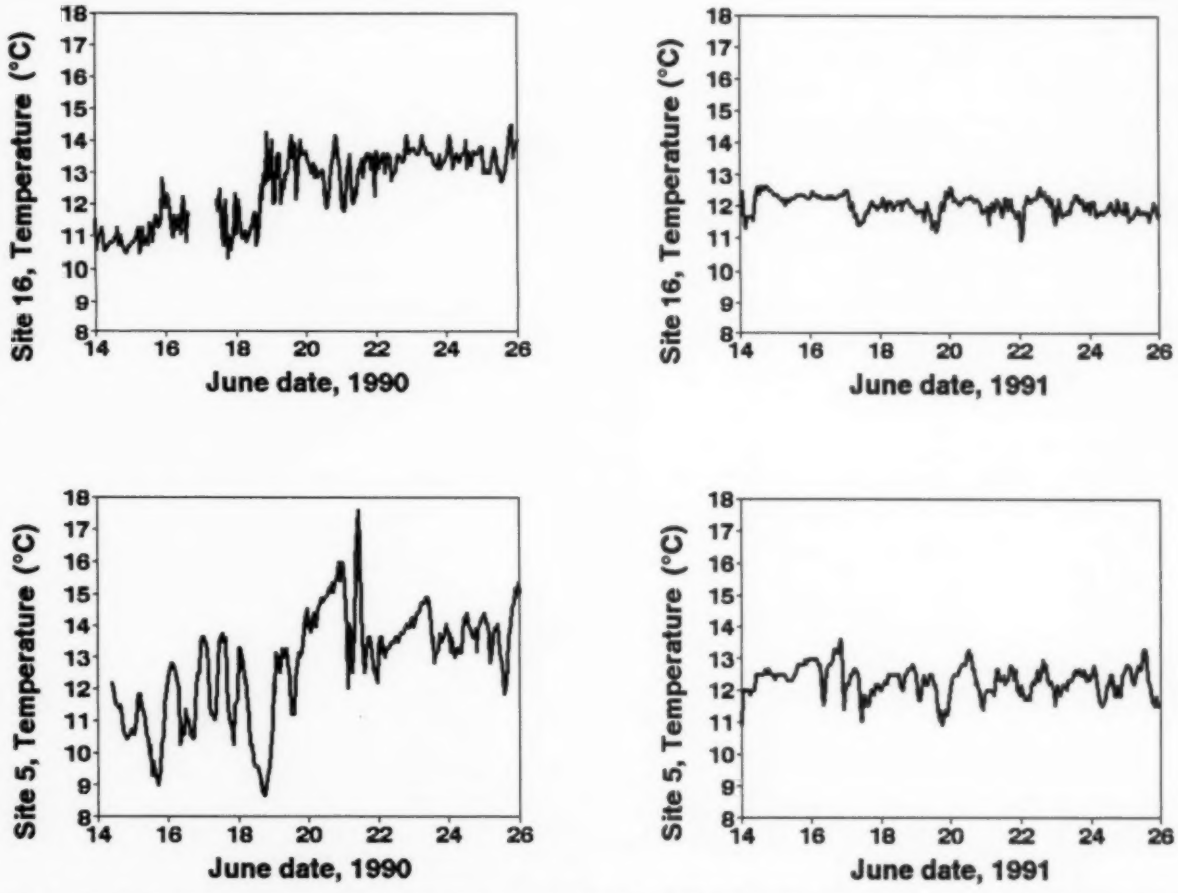

FIG. 1. Temperatures (at 9 m) at two remote yellow perch spawning sites (5 and 16) in Labrador Lake during the embryo development period (June 14–26) for the years 1990 and 1991.

would cause the embryos to frequently experience relatively large temperature swings or temperature shocks. Temperature reversals or shocks experienced during early embryonic development have a greater potential to produce larval malformations and/or subsequent deaths than such temperature regimes during the later embryonic stages (Murray and Beacham 1987).

The overlap of the period of the wind index providing the smallest SSE for each site and the pelagic phase of Lochaber yellow perch is quite small, but it may occur during this important developmental stage. The length of the overlap is 0–10 d.

A possible effect of high winds during the pelagic phase could be to uniformly mix the planktonic food utilized by the perch larvae (Peterman and Bradford 1987) which in turn may result in starvation. However, Hokanson and Kleiner (1974) noted that some free-swimming larvae can survive for 15 d in the absence of food. Thus, winds during the last 0-10 d of June may not have as great an impact on the survival of the YOY as winds during the early part of June.

The temperature recordings obtained at two isolated perch spawning sites (Sites 5 and 16) in our study lake in 1990 and 1991 indicate that interannual and intersite heterogeneity in temperature regimes occurs. Such heterogeneities may explain the differences in survivorship of embryos that develop at those sites in different years.

Based on our investigations, we suggest that winds prior to and during embryonic development may kill or, more likely, result in deformed larvae due to frequent temperature shocking. Such deformed larvae may subsequently starve because they are unable to feed at an appropriate rate, or they may not develop adequately to avoid predation. The effects of the winds on embryonic perch vary with spawning site and thus can produce independently varying demes of yellow perch in Lochaber Lake.

We do not expect all yellow perch populations to be affected by winds in the same way as those in Lochaber. Lochaber is a relatively long, narrow, and deep lake. In shallow lakes, severe winds may cause perch embryos and larvae to experience physical damage rather than temperature shocking. In deeper lakes, thermoclines may form prior to or during the spawning period, and yellow perch may spawn at depths near the thermocline. Under such conditions, perch embryos may experience frequent thermal shocking and subsequent mortality due to wind-driven internal seiches. Demes defined by spawning sites in such lakes may vary independently because winds differentially affect each deme.

Acknowledgements

We wish to thank A. Tompkins, B. MacNeil, K. MacCormick, and M. MacLaughlin for their field assistance. R. Lewis provided assistance with site surveys. The Natural Sciences and Engineering Research Council of Canada and the University Council for Research, St. Francis Xavier University, provided financial support for this work.

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Appendix

Models relating the egg-mass counts c_t in year t to the counts in other years can be developed from the Leslie matrix model for a population of female yellow perch. If $n_{x,t}$ denotes the number of female yellow perch of age x at time t, $p_{0,t}$ is the time-varying probability of survival from age 0 to age 1, p_x $(x > 0)$ is the age-specific probability of survival, and m_x is the age-

specific fecundity ($x > 3$ for Lochaber yellow perch), then (Aalto and Newsome 1980)

$$n_{0,t} = \sum_{x=3}^{k} m_x n_{x,t}$$

$$n_{1,t} = p_{0,t-1} n_{0,t-1}$$

$$n_{x,t} = p_x n_{x-1,t-1}, \qquad x \geqslant 2.$$

Algebraic manipulation yields the following equation:

$$\begin{aligned}\sum_{x=3}^{k} n_{x,t+3} = p_1 p_2 [&p_{0,t} m_3 n_{3,t} \\ &+ \sum_{x=4}^{k} (p_{0,t} m_x + p_{0,t+3-x} m_3) n_{x,t}] \\ &+ p_1 p_2 p_3 [p_{0,t-1} m_4 n_{4,t-1} \\ &+ \sum_{x=5}^{k} (p_{0,t-1} m_x + p_{0,t+3-x} m_4) n_{x,t-1}] \\ &+ \ldots + p_{0,t+3-k} p_1 p_2 \cdots p_{k-1} m_k n_{k,t+3-k}.\end{aligned}$$

The coefficients of $n_{x,t}$, $n_{x,t-1}$, etc. that are sums of two terms will vary less over time than those containing only one term (sums of random variables usually vary less than the individual summands). The sums multiplied by $p_1p_2p_3$, $p_1p_2p_3p_4$, etc. will vary less in magnitude than the first term because they are multiplied by numbers less than 1 in magnitude (the p_j's). The major source of variation is $p_1p_2p_{0,t}m_3n_{3,t}$, and as a first approximation, we replace all other terms by a constant E to obtain the following model:

$$\sum_{x=3}^{k} n_{x,t+3} = p_1 p_2 p_{0,t} m_2 n_{3,t} + E.$$

If the age 3 year-class is a significant portion of the population, then the summation on the left may be approximated by $n_{3,t+3}$ to obtain the following model:

$$n_{3,t+3} = p_1 p_2 p_{0,t} n_{3,t} + E.$$

Assume

$$p_{0,t} = e^{-(Z + \mathrm{BW}_t)}$$

where Z is the instantaneous rate of mortality due to other causes and BW_t is the instantaneous rate of mortality due to the wind index W_t (Pope 1973). Assuming that Z varies much less than BW_t, we obtain the following model:

$$n_{3,t+3} = Ae^{-\mathrm{BW}_t} n_{3,t} + E.$$

Finally, because the yearly egg-mass counts are proportional to the number of female yellow perch using a deme, we replace $n_{3,t}$ by c_t to obtain the following model:

$$c_{t+3} = Ae^{-\mathrm{BW}_t} c_t + E.$$

This model allows us to study what may be the most variable feature of the environment of the yellow perch, i.e. the winds prior to and during the embryonic development period.

Similar arguments may be used to develop the following model relating the egg-mass counts at times $(t + 3)$, t, and $(t - 1)$:

$$c_{t+3} = AE^{-\mathrm{BW}_t} c_t + De^{-\mathrm{BW}_{t-1}} c_{t-1} + E.$$

Effects of Nutritional Regime on Correlates of Growth Rate in Juvenile Atlantic Cod (*Gadus morhua*): Comparison of Morphological and Biochemical Measurements

A. R. Foster[1]

Zoology Department, Aberdeen University, Aberdeen, Scotland, U.K., AB9 2TN

and

SOAFD Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, Scotland, U.K., AB9 8DB

D.F. Houlihan

Zoology Department, Aberdeen University, Aberdeen, Scotland, U.K., AB9 2TN

and S. J. Hall

SOAFD Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, Scotland, U.K., AB9 8DB

Foster, A. R., D. F. Houlihan, and S. J. Hall. 1993. Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod (*Gadus morhua*): comparison of morphological and biochemical measurements. Can. J. Fish. Aquat. Sci. 50: 502–512.

The effects of both long-term (35 d of daily feeding, feeding every second day, or starvation) and short-term (up to 15 d of refeeding following starvation) nutritional regimes on morphological (tissue-somatic indices) and biochemical measurements (RNA concentration, RNA/protein ratio, RNA/DNA ratio, and cytochrome c oxidase activity (CCO)) were investigated for a variety of Atlantic cod (*Gadus morhua*) tissues. Liver-somatic index was the morphological index most sensitive to both the long- and short-term nutritional regimes. The majority of the tissue RNA measurements demonstrated positive linear relationships with growth rate, although stomach, intestine, and white muscle were the most sensitive tissues for all the treatments. Most of the tissues examined also showed positive linear relationships with growth rate, although stomach, intestine, and white muscle were the most sensitive tissues for all the treatments. Most of the tissues examined also showed positive linear relationships between CCO specific activity and long-term growth rate. However, tissue CCO specific activity was relatively insensitive to the short-term alterations in nutritional regime.

Les auteurs ont étudié les effets de régimes alimentaires à long terme (35 jours d'alimentation quotidienne, d'alimentation à tous les deux jours ou de privation) et à court terme (jusqu'à 15 jours d'alimentation après privation) sur certains paramètres morphologiques (indices somatiques tissulaires) et biochimiques (concentration d'ARN, rapport ARN/protéines, rapport ARN/ADN et activité de la cytochrome c oxydase (CCO)) dans divers tissus de la morue. L'indice somatique hépatique s'est avéré l'indice morphologique le plus sensible aux deux types de régimes alimentaires. La plus grande partie des dosages du RNA tissulaire présentait une relation linéaire positive avec le taux de croissance, mais ce sont les tissus de l'estomac, de l'intestin et des muscles lisses qui étaient les plus sensibles à tous les traitements. La plupart des tissus examinés présentaient aussi des relations linéaires positives entre l'activité spécifique de la CCO et le taux de croissance à long terme. L'activité spécifique de la CCO tissulaire était cependant relativement peu sensible aux modifications à court terme du régime alimentaire.

Received April 3, 1992
Accepted September 17, 1992
(JB460)

Reçu le 3 avril 1992
Accepté le 17 septembre 1992

Various indices have been used to evaluate the nutritional condition and growth rate of fish. Growth rate (nutritional status) indices can be categorised under two main headings: morphological and biochemical. Morphological indices include the liver-somatic index (Jensen 1979), body water content (Elliott 1976), and condition factor (Bulow 1970). Biochemical indices include tissue nucleic acid concentrations/ratios (Buckley 1979; Houlihan et al. 1989), ribosomal capacity (Lied and Rosenlund 1984), and aerobic enzyme activities (Smith and Chong 1982; Goolish and Adelman 1987). Of the biochemical indices, tissue RNA concentrations/ratios and cytochrome *c* oxidase (CCO) activity are increasingly used as indices of growth rate, as these parameters are positively correlated with rates of protein synthesis and whole-body growth rate in a variety of fish species (Houlihan et al. 1989; Foster 1990; Foster et al. 1991; Mathers et al. 1992).

However, the majority of studies investigating the relationship between these parameters and whole-body growth rate have concentrated on the effects of long-term (typically $\geq$4 wk) nutritional treatment, whilst there has been little attempt to describe short-term nutritional effects on the above indices. Investigation of short-term responses is important because the speed of response of any index to a change in nutrition must be

[1]Present address: Rowett Research Institute, Bucksburn, Aberdeen, Scotland, U.K., AB2 9SB.

TABLE 1. Summary of the treatment groups, initial and final body weights, length of the experimental period, and weight-specific growth rate (corrected for body size) of the fish. The water temperature was 10 ± 0.5°C and the photoperiod was 13 h dark : 11 h light. Values are mean ± 1 SEM and sample sizes are given. Statistical comparisons were performed using one-way ANOVA, and where the ANOVA was significant, differences between means were tested using the Student–Newman–Keuls multiple comparison procedure (Zar 1974). Significance was taken at $p \leq 0.05$ and an asterisk denotes a significant difference between the starved group and any other treatment group. ND = not determined.

Treatment group	Initial body weight (g)	Final body weight (g)	Experimental period (d)	Specific growth rate (%·d^{-1})
I. Daily (n = 12)	310.2 ± 28.2*	465.5 ± 43.8*	35	1.21 ± 0.09*
II. Alternate (n = 12)	270.9 ± 15.0	357.7 ± 23.1*	35	0.64 ± 0.13*
III. Starved (n = 10)	254.5 ± 17.0	204.2 ± 16.6	35	−0.66 ± 0.06
IV. Refed (n = 12)	303.5 ± 10.7*	ND	5–15	ND

TABLE 2. Linear regressions of tissue-somatic indices against body size corrected, whole-body weight-specific growth rate (% change in wet weight·d) for treatment groups I–III. Y = tissue-somatic index and X = weight-specific growth rate. The tissue-somatic indices were expressed as % wet body weight. The linear regressions were tested for statistical significance using ANOVA. The coefficient of determination (r^2) is shown for each regression. The sample size was 34 for each tissue. NS denotes a statistically insignificant linear relationship ($p > 0.05$).

Tissue	Linear regression equation	r^2	p value
Ventricle	$Y = 0.082 - 0.002X$	0.012	NS
Stomach	$Y = 1.600 - 0.079X$	0.176	<0.02
Intestine	$Y = 0.739 - 0.060X$	0.167	<0.02
Liver	$Y = 3.800 + 2.800X$	0.872	<0.001

known before the value of a parameter as a measure of growth rate (nutritional status) can be properly assessed. The investigation of short-term responses is also particularly important if growth rate correlates are to be used as indicators of water pollution where the exposure time to a pollutant may be short (Kearns and Atchison 1979; Barron and Adelman 1984).

Although tissue-somatic indices, RNA concentrations/ratios, and aerobic enzyme activities have been used as indices of growth rate (nutritional status) of fish in separate studies, to our knowledge, this paper is the first to compare all three sets of parameters concurrently. The aims of the present study are first to describe the long-term effects (>4 wk) of nutritional regime (i.e. 35 d of daily feeding, feeding every second day, or starvation) on tissue-somatic indices, nucleic acid and protein concentrations/ratios, and CCO activity in juvenile Atlantic cod (*Gadus morhua*), and second to investigate the time course of these morphological and biochemical measurements with refeeding after a period of starvation. This information will allow us to determine which variate(s) and which tissue(s) are the most sensitive indicators of growth rate (nutritional status) in cod exposed to different nutritional regimes.

Methods and Materials

Maintenance of Animals and Experimental Groups

Juvenile Atlantic cod (approximately 1 yr old) were obtained from Millport Marine Station, Isle of Cumbrae, and maintained at SOAFD Marine Laboratory in circular stock tanks (2 m in diameter) for at least 4 wk. During this period the fish were fed sandeels (*Ammodytes* spp.) ad libitum and were maintained under ambient water temperature (10 ± 0.5°C) and photoperiod (13 h dark : 11 h light). Following this acclimation period, fish were starved for 2 d to ensure the complete evacuation of gut contents. The fish were then anaesthetised, weighed, freeze-branded for identification purposes, and randomly allocated to one of the five treatments (Table 1) (10 or 12 fish were assigned to each treatment, Table 1). The fish in treatment group I were fed daily; those in treatment group II were fed on alternate days. This regime was continued for 35 d. Fish in groups I and II were offered sandeels (ration size ~8% of body weight, i.e. 300 ± 30 g per treatment group per feeding) and any food remaining after 20 min was removed. The cod in treatment group III were starved for 35 d. The fish in treatment group IV were starved for 35 d and then refed sandeels ad libitum for the following 15 d. In this group, day 0 represents the first day following starvation. These fish were sampled at 5, 11, and 15 d after refeeding began. With the exception of the fish that were fed daily (treatment group I, which was maintained in a 2-m-diameter circular tank), the fish were held in pairs in 70-L aquaria (35 × 65 cm) in which the water was continuously aerated. During the experiment the water temperature and photoperiod were fixed at 10 ± 0.5°C and 13 h dark : 11 h light.

At the time of each sampling, the fish were killed by a sharp blow to the head and transection of the spinal cord. The fish were then weighed, rapidly dissected on ice, and the following tissues (or subsamples of tissues) dissected out and weighed where appropriate: total heart, ventricle, liver, stomach, intestine, gill, and a subsample of white muscle (from the dorsolateral region of the fish). All tissues were frozen in liquid nitrogen and stored at −70°C until analysis. Any gut contents were removed, weighed and subtracted from the weight of the fish.

Using the dissection data, tissue-somatic indices were calculated as

$$\text{Tissue-somatic index (\% wet body weight)} = \frac{\text{tissue weight (g)}}{\text{(whole fish weight} - \text{gut contents (g))}} \times 100.$$

Protein, RNA, and DNA Determinations

Tissue protein and RNA concentrations were determined on the same tissue sample after the homogenization of 100 mg of tissue in 0.5 M PCA as described by Foster et al. (1991, 1992a, 1992c). Protein content was determined by the technique of Lowry et al. (1951), as modified by Schacterle and Pollack (1973). RNA was extracted by the Scmidt–Thannhauser procedure (Munro and Fleck 1966) and its concentration measured using the orcinol method of Mejbaum (1939), with calf liver RNA as standard. Tissue nucleic acid and protein concentrations/ratios were expressed as RNA/DNA (micrograms RNA/

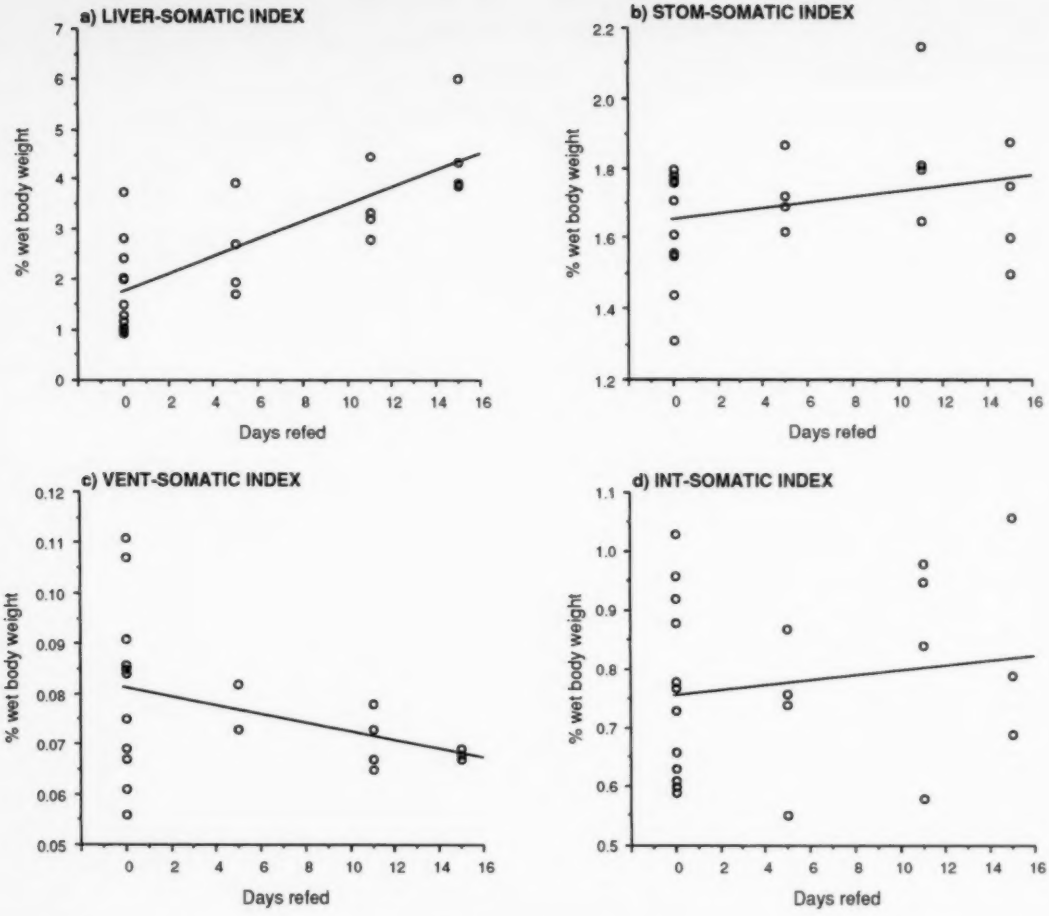

FIG. 1. Linear regressions of tissue-somatic indices against number of days refed for the pooled treatment groups III and IV. Y = tissue-somatic index and X = number of days refed. The tissue-somatic indices are expressed as % wet body weight. The linear regressions were tested for statistical significance using ANOVA and the results are given below. The coefficient of determination (r^2) and probability values are shown for each regression. The sample size was 22 for each tissue. NS denotes an insignificant linear relationship ($p > 0.05$). Linear relationships: (a) liver, $Y = 1.76 + 0.17X$, $r^2 = 0.615$, $p < 0.001$; (b) stomach (STOM), $Y = 1.650 + 0.008X$, $r^2 = 0.087$, NS; (c) ventricle (VENT), $Y = 0.081 - 0.001X$, $r^2 = 0.179$, $p < 0.05$; (d) intestine (INT), $Y = 0.756 + 0.004X$, $r^2 = 0.027$, NS.

micrograms DNA), RNA/protein (micrograms RNA/milligrams protein), RNA concentration (micrograms RNA per gram tissue), DNA concentration (micrograms DNA per milligram tissue, an index of cell number (Winick and Noble 1965, 1966)), protein concentration (milligrams protein per gram tissue), and protein/DNA ratio (micrograms protein/micrograms DNA, an index of cell size (Winick and Noble 1965, 1966)).

CCO: Tissue Preparation and Assay

Frozen tissues samples (30–100 mg) were weighed and disrupted in ice-cold extraction buffer using a sonicator to release CCO from the mitochondria. The extraction buffer contained 50 mM Tris–HCl, 75 mM sucrose, 200 mM mannitol, 2 mM $MgCl_2 \cdot 6H_2O$), 5 mM EDTA, 1 mM dithiothreitol (Hansen and Sidell 1983), and 0.1 mM phenylmethyl sulphonyl fluoride (PMSF, general protease inhibitor). The extraction buffer was then adjusted to pH 7.6 at 20°C. A time course of sonication was performed on each tissue to obtain optimal enzyme release and activity.

Sonicates were centrifuged at 13 000 *g* for 2 min and the ice-cold supernatant was used for CCO measurement. For liver, samples of supernatant were taken from beneath a lipid layer found in all liver samples.

The spectrophotometric assay for CCO (EC 1.9.3.1) was based on the oxidation of cytochrome *c*. The rate of CCO activity was measured in duplicate at 10°C by the decline in absorbance at 550 nm using 0.065% (w/v) cytochrome *c* in 50 mM phosphate buffer. The reaction was initiated by the addition of ice-cold enzyme supernatant. Preliminary experiments were performed to verify that the enzyme activities were measured under saturating conditions and to determine the pH optima for CCO activity in each of the tissues. CCO activity was calculated using a millimolar extinction coefficient of 18.5; CCO

specific activities were expressed as micromoles cytochrome *c* oxidised per minute per gram tissue.

Whole-Body Growth Rates, RNA, CCO, and Body Size Correction

Whole-body, weight-specific growth rates were calculated using the following equation (Ricker 1979):

$$\text{Specific growth rate } (\%\cdot d^{-1}) = \frac{(\log_e W_2 - \log_e W_1)}{t} \times 100$$

where W_1 and W_2 are the initial and final weights (grams) of the experimental fish, respectively, and t is the length of the experimental period (days).

Owing to the range in initial and final body weights between the treatment groups (Table 1), weight-specific growth rates, tissue CCO specific activities, and RNA concentrations of the continuously fed fish (treatments I and II) were corrected for body size to a standard 400-g fish (approximate average final weight of fed fish) using the $\log_e$ transformation of the allometric equation $Y = aX^b$ with an exponent of -0.441 (growth rate) or -0.2 (CCO specific activity) (Jobling 1983; Houlihan et al. 1988; Foster et al. 1992c; Mathers et al. 1992). These parameters were only corrected for body size for the two groups of fish that were fed continuously (treatment groups I and II), as scaling relationships have only been reported from experiments with continuously fed fish (Mathers et al. 1992).

Statistical Analysis

Homogeneity of variances between treatment groups were tested using one-way analysis of variance (ANOVA), and where the ANOVA was significant, differences between means were tested using the Student–Newman–Keuls multiple comparison procedure (Zar 1974). Linear regressions were tested for significance using ANOVA. Statistical differences between the gradients of regression equations were tested using the modified Student *t*-statistic, and statistical significance was taken at $p \leq 0.05$.

Results

Effects of Nutritional Regime on Whole-Body Growth Rate and Tissue-Somatic Indices

Weight-specific growth rates were increased significantly with daily feeding, compared with feeding on alternate days, and were negative in the starved group (Table 1).

Table 2 shows the relationships between tissue-somatic index and long-term whole-body growth rate for treatment groups I–III. It can be seen that the liver-somatic index was strongly dependent on growth rate, as more than 87% of the variability in the liver-somatic index was accounted for by growth rate. The liver-somatic index also had the steepest gradient of all the tissue-somatic indices that were measured. Negative linear relationships with low gradients were observed for the stomach- and intestine-somatic indices, whilst no significant linear relationship was observed between the ventricle-somatic index and whole-body growth rate.

Figure 1 illustrates the effects of refeeding following 35 d of starvation on the tissue-somatic indices of cod. In these figures the data from the starved group (treatment group III) and the refed group (treatment group IV) have been pooled for the calculation of the linear regressions. It can be seen that liver was the only tissue that showed a significant positive relationship with refeeding; it also had the steepest gradient. In contrast with the statistically insignificant relationship for ventricle shown in Table 2, with refeeding, the ventricle-somatic index gave a significantly negative relationship with a very low gradient. The stomach- and intestine-somatic indices were independent of refeeding.

TABLE 3. Linear regressions of tissue RNA measurements (tissue RNA concentration (μg RNA·g tissue^{-1}), RNA/protein ratio (μg RNA/mg protein), RNA/DNA ratio (μg RNA/μg DNA)) against weight-specific growth rate (% change in wet weight·d^{-1}, corrected for body size) for treatment groups I–III. Y = RNA parameter and X = weight-specific growth rate. The linear regressions were tested for statistical significance using ANOVA and the results are given below. The coefficient of determination (r^2) is also shown for each regression. The sample size was 34 for each tissue.

Tissue	Linear regression equation	r^2	p value
White muscle			
RNA	$Y = 934 + 348X$	0.755	<0.001
RNA/protein	$Y = 6.33 + 1.96X$	0.660	<0.001
RNA/DNA	$Y = 3.14 + 1.31X$	0.740	<0.001
Ventricle			
RNA	$Y = 2051 + 157X$	0.177	<0.02
RNA/protein	$Y = 16.0 + 1.21X$	0.128	<0.05
RNA/DNA	$Y = 1.04 + 0.122X$	0.375	<0.001
Stomach			
RNA	$Y = 1976 + 520X$	0.398	<0.001
RNA/protein	$Y = 16.3 + 3.79X$	0.281	<0.001
RNA/DNA	$Y = 0.903 + 0.257X$	0.408	<0.001
Intestine			
RNA	$Y = 3700 + 857X$	0.491	<0.001
RNA/protein	$Y = 28.2 + 7.75X$	0.505	<0.001
RNA/DNA	$Y = 1.82 + 0.610X$	0.500	<0.001
Gill			
RNA	$Y = 3490 + 439X$	0.455	<0.001
RNA/protein	$Y = 34.4 + 3.04X$	0.213	<0.001
RNA/DNA	$Y = 0.732 + 0.095X$	0.302	<0.005

Effects of Nutritional Regime on Protein, DNA, and RNA Concentrations and Ratios

In all four treatment groups, tissue protein concentrations were unaffected by the treatments and there were no significant relationships with growth rate (Foster 1990). The one exception to this was white muscle where there was a positive linear relationship between protein concentration (Y, milligrams protein per gram tissue) and whole-body growth rate (X, percent wet body weight per day) ($Y = 147 + 9.16X$, $r^2 = 0.247$, $p < 0.005$).

Tissue DNA concentration (index of cell number) and protein/DNA ratios (index of cell size) were largely unaffected by any of the treatments (Foster 1990). However, a positive linear relationship was observed between white muscle cell size (Y, micrograms protein/micrograms DNA) and growth rate ($Y = 492 + 48X$, $r^2 = 0.23$, $p < 0.001$).

Within treatment groups I–III, tissue RNA concentration, RNA/protein ratio, or RNA/DNA ratio was generally similar between treatment groups I and II but significantly higher when compared with the starved group (Foster 1990). Table 3 presents the linear relationships that were observed between the tissue RNA measurements (e.g. RNA concentration, RNA/protein ratio, and RNA/DNA ratio) and weight-specific, whole-

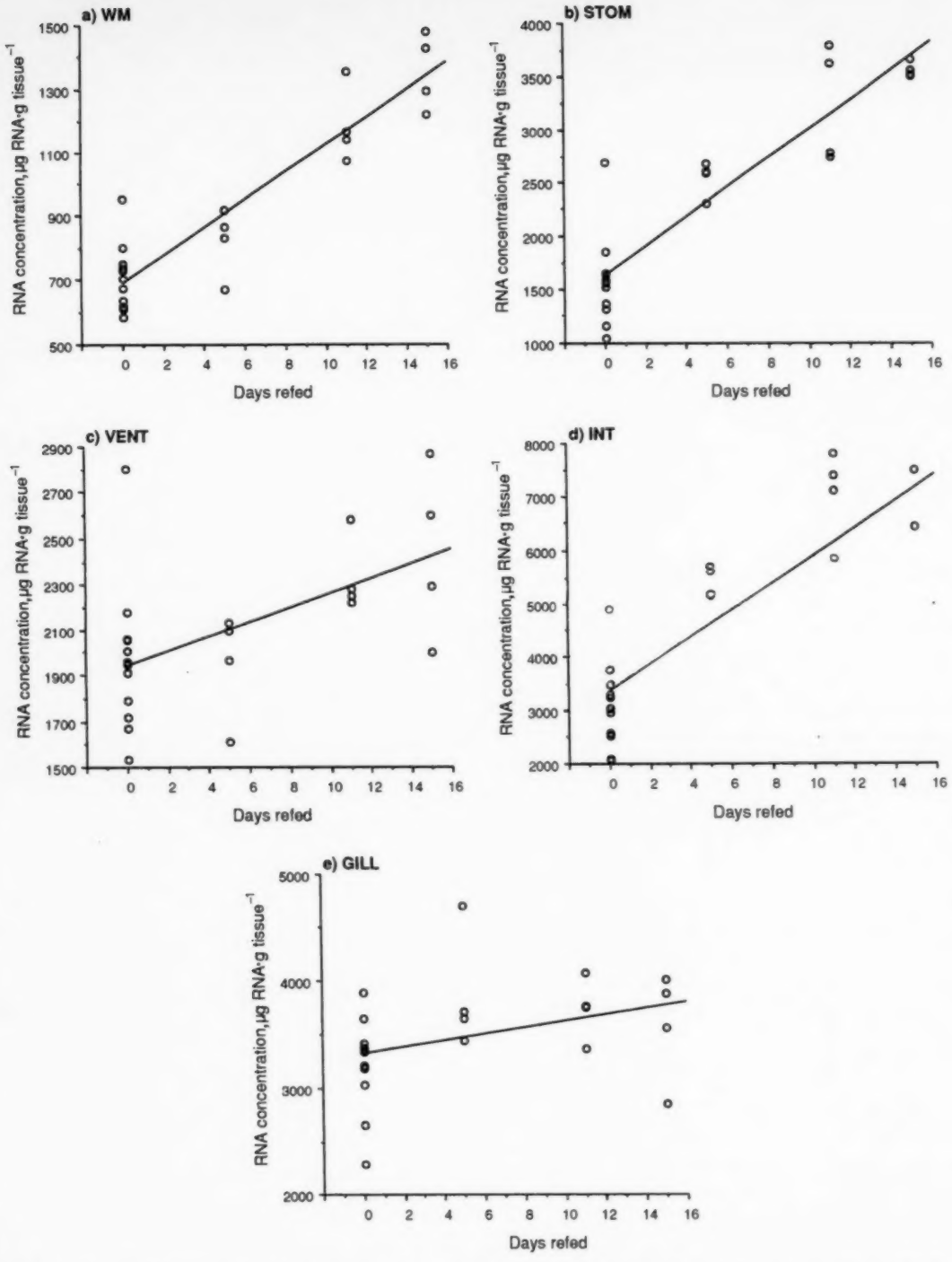

FIG. 2. Linear regressions of RNA concentration against the number of days refed for the pooled treatment groups III and IV. Y = RNA concentration and X = number of days refed. The linear regressions were tested for statistical significance using ANOVA and the results are given below. The coefficient of determination (r^2) and probability values are also shown for each regression. The sample size was 22 for each tissue. NS denotes a statistically insignificant linear relationship. Linear relationships: (a) white muscle (WM), $Y = 690 + 43.3X$, $r^2 = 0.857$, $p < 0.001$; (b) stomach (STOM), $Y = 1636 + 138X$, $r^2 = 0.832$, $p < 0.001$; (c) ventricle (VENT), $Y = 1943 + 31.9X$, $r^2 = 0.309$, $p < 0.005$; (d) intestine (INT), $Y = 3353 + 256X$, $r^2 = 0.720$, $p < 0.001$; (e) gill, $Y = 3334 + 29.6X$, $r^2 = 0.131$, NS.

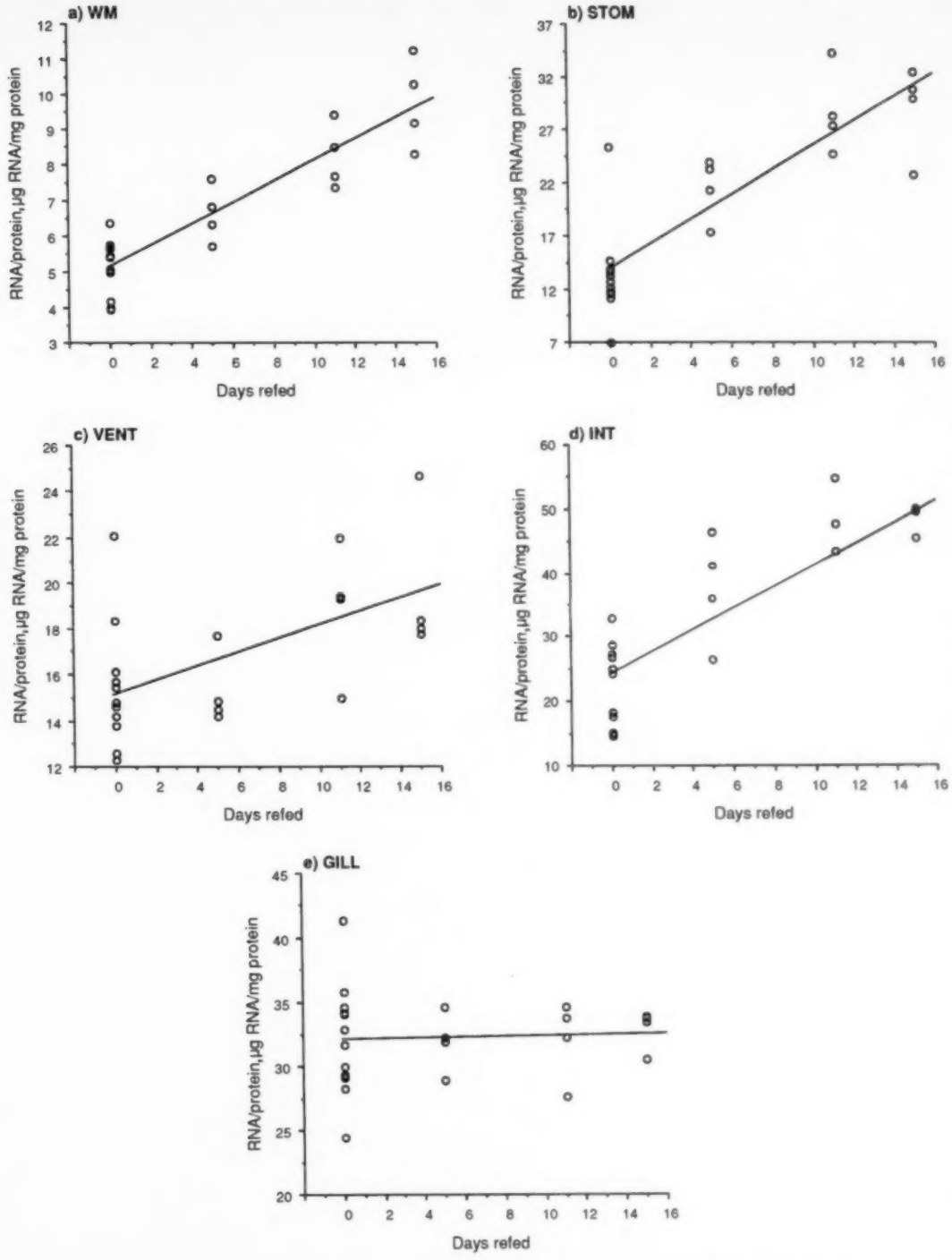

FIG. 3. Linear regressions of tissue RNA/protein ratio against the number of days refed for the pooled treatment groups III and IV. Y = RNA/protein ratio and X = number of days refed. The linear regressions were tested for statistical significance using ANOVA and the results are given below. The coefficient of determination (r^2) and probability values are also shown for each regression. The sample size was 22 for each tissue. NS denotes a statistically insignificant linear relationship. Linear relationships: (a) white muscle (WM), $Y = 5.17 + 0.30X$, $r^2 = 0.820$, $p < 0.001$; (b) stomach (STOM), $Y = 13.9 + 1.15X$, $r^2 = 0.743$, $p < 0.001$; (c) ventricle (VENT), $Y = 15.2 + 0.30X$, $r^2 = 0.328$, $p < 0.005$; (d) intestine (INT), $Y = 24.4 + 1.69X$, $r^2 = 0.688$, $p < 0.001$; (e) gill, $Y = 32.1 + 0.03X$, $r^2 = 0.003$, NS.

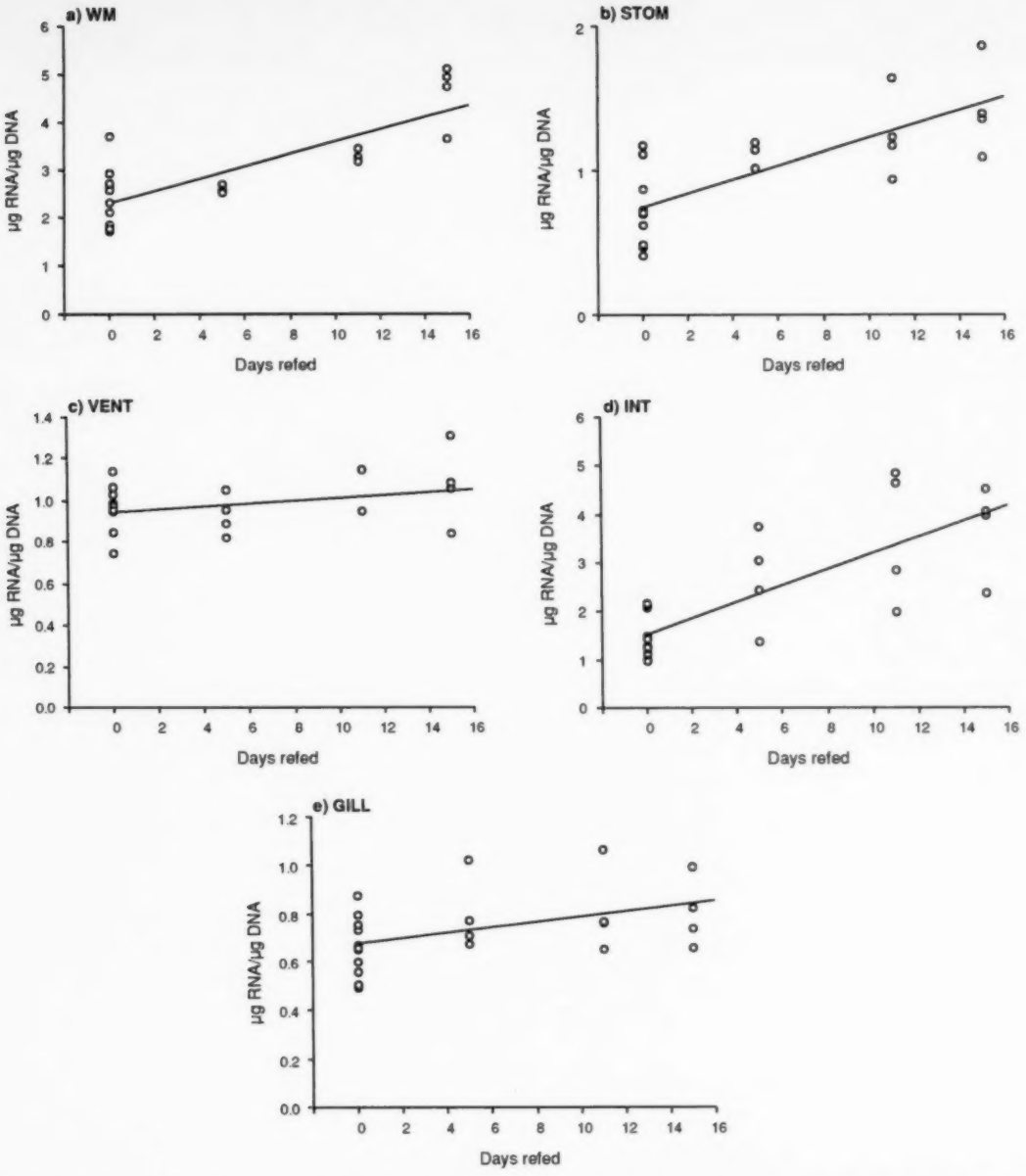

FIG. 4. Linear regressions of tissue RNA/DNA ratio against number of days refed for the pooled treatment groups III and IV. Y = RNA/DNA ratio and X = number of days refed. The linear regressions were tested for statistical significance using ANOVA and the results are given below. The coefficient of determination (r^2) and probability values are also shown for each regression. The sample size was 22 for each tissue. NS denotes a statistically insignificant linear relationship. Linear relationships: (a) white muscle (WM), $Y = 2.30 + 0.13X$, $r^2 = 0.661$, $p < 0.001$; (b) stomach (STOM), $Y = 0.737 + 0.05X$, $r^2 = 0.596$, $p < 0.001$; (c) ventricle (VENT), $Y = 0.945 + 0.01X$, $r^2 = 0.110$, NS; (d) intestine (INT), $Y = 1.54 + 0.17X$, $r^2 = 0.613$, $p < 0.001$; (e) gill, $Y = 0.676 + 0.011X$, $r^2 = 0.200$, $p < 0.05$.

body growth rate. For every tissue examined, a positive linear relationship was observed between each RNA measurement and long-term whole-body growth rate. However, for most of the RNA measurements, the intestine and stomach were the most sensitive tissues to changes in whole-body growth rate (i.e. demonstrated the steepest gradients).

With daily ad libitum refeeding following 35 d of starvation, most of the tissue RNA concentrations and RNA/protein ratios increased with refeeding, with intestine and stomach consistently having the steepest gradients ($p < 0.05$) (Fig. 2 and 3). The majority of the tissue RNA/DNA ratios also increased with refeeding, with intestine, white muscle, and stomach having the steepest gradients (Fig. 4). In contrast, ventricle RNA/DNA was independent of refeeding. For gill, a significant linear relationship was only observed between the gill RNA/DNA ratio and the duration of refeeding.

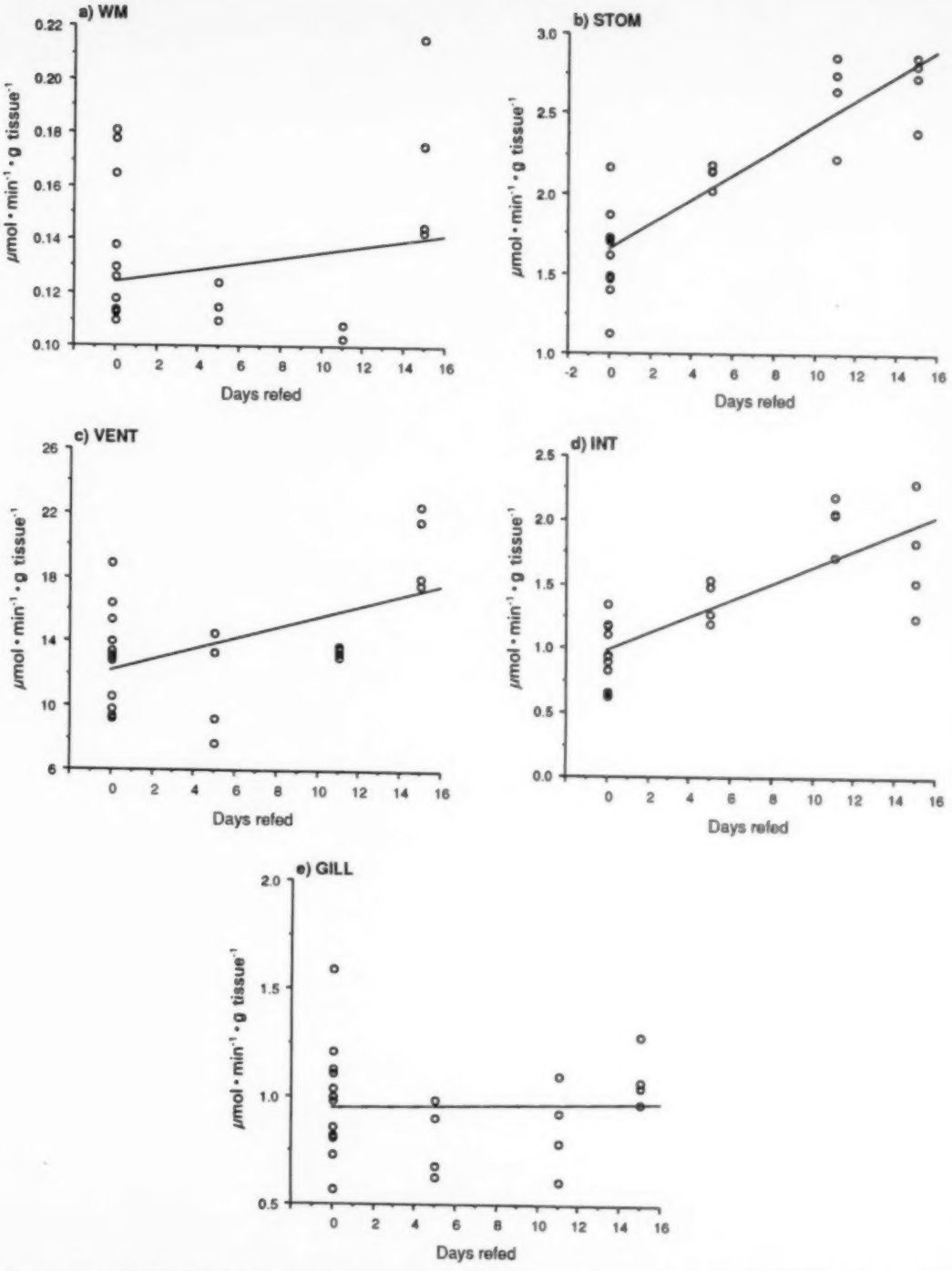

FIG. 5. Linear regressions of CCO activity against the number of days refed for pooled treatment groups III and IV. Y = tissue CCO specific activity and X = number of days refed. The linear regressions were tested for statistical significance using ANOVA and the values are given below. The coefficient of determination (r^2) and probability values are also shown for each regression. The sample size was 22 for each tissue. NS denotes a statistically insignificant linear relationship ($p \leq 0.05$). Linear relationships: (a) white muscle (WM), $Y = 0.124 + 0.001X$, $r^2 = 0.042$, NS; (b) stomach (STOM), $Y = 1.65 + 0.078X$, $r^2 = 0.798$, $p < 0.001$; (c) ventricle (VENT), $Y = 12.2 + 0.33X$, $r^2 = 0.286$, $p < 0.01$; (d) intestine (INT), $Y = 0.984 + 0.066X$, $r^2 = 0.642$, $p < 0.001$; (e) gill, $Y = 0.944 + 0.002X$, $r^2 = 0.200$, NS.

TABLE 4. Linear regressions of CCO activity ($\mu mol \cdot min^{-1} \cdot g\ tissue^{-1}$) against body size corrected, weight-specific growth rate (% change in wet weight·d^{-1}) for treatment groups I–III. Y = tissue CCO specific activity and X = weight-specific growth rate. The linear regressions were tested for statistical significance using ANOVA and the values are given below. The coefficient of determination (r^2) is also shown for each regression. The sample size was 34 for each tissue. NS denotes a statistically insignificant linear relationship.

Tissue	Linear regression equation	r^2	p value
White muscle	$Y = 0.153 + 0.033X$	0.215	<0.01
Ventricle	$Y = 14.50 + 2.17X$	0.241	<0.005
Stomach	$Y = 1.94 + 0.44X$	0.595	<0.001
Intestine	$Y = 1.31 + 0.43X$	0.445	<0.001
Gill	$Y = 0.936 + 0.002X$	0.000	NS

Reproducible liver RNA, DNA, and protein determinations were not obtained, presumably because of the large amounts of lipid in this tissue. Liver samples were therefore omitted from the analyses.

Effects of Nutritional regime on CCO Activity

For treatment groups I–III, the majority of the tissue CCO specific activities were similar between treatment groups I and II. CCO specific activities were also higher in treatment group I (daily-fed group) compared with the starved fish (treatment group III, Foster 1990).

Positive linear relationships were observed between most tissue CCO specific activities (micromoles per minute per gram tissue) and long-term whole-body growth rate (Table 4). Ventricle, stomach, and intestine all demonstrated steep gradients ($p < 0.05$) compared with white muscle. However, the gradient of the linear relationship between ventricle CCO specific activity and growth rate was significantly greater ($p < 0.01$) than that observed for the stomach and intestine CCO specific activities. In contrast, gill CCO specific activity was independent of growth rate.

Figure 5 illustrates the effects of refeeding on tissue CCO specific activities. It can be seen that stomach, intestine, and ventricle CCO specific activities increased linearly with time following refeeding, with ventricle having the steepest gradient ($p < 0.01$). However, significant linear relationships were not observed between white muscle and gill CCO specific activities and the number of days refed.

Reproducible liver CCO specific activity was not observed and liver CCO measurement was therefore omitted from the analyses.

Discussion

Relationships between Tissue Somatic Indices and Whole-Body Growth Rate

A positive linear relationship was only observed between liver-somatic index and growth rate (Table 2). Changes in the liver-somatic index were also the most sensitive to refeeding following 35 d of starvation (Fig. 1a). In contrast, the stomach- and intestine-somatic indices decreased with increasing growth rate (Table 2) and demonstrated relatively low gradients. The ventricle-somatic index was independent of growth rate. With the exception of liver, these other tissues also showed little response to refeeding following 35 d of starvation (Fig. 1).

Black and Love (1986) examined the sequence of mobilisation of energy reserves in cod and observed that in the early stages of starvation (approximately 10 wk), liver lipids and the glycogens of liver and white muscle were utilised first. As gadoids, such as cod, store lipid almost exclusively in the liver (Black and Love 1986), the observed rapid response of this tissue to the nutritional regimes is presumably the result of liver lipid storage during periods of growth and refeeding and liver lipid mobilisation (in the form of free fatty acids) and its subsequent oxidation via the β-oxidation pathway during periods of starvation.

Relationships between Nucleic Acid/Protein Data, CCO Activity, and Whole-Body Growth Rate

It is well documented that feeding results in increased rates of protein synthesis and growth in fish (Houlihan et al. 1988). However, the maximal rate of growth will depend upon an organism's total capacity for protein synthesis which in turn will be a function of RNA concentration/activity. As protein synthesis is obviously an energy-consuming process, it is thus not surprising that aerobic capacity (CCO activity and concomitant ATP production) and RNA content are both positively correlated with protein synthetic capacity and growth.

For every tissue examined, the RNA measurements (i.e. RNA concentration, RNA/protein ratio, and RNA/DNA ratio) demonstrated positive linear relationships with whole-body growth rate (Table 3). For most of the RNA measurements, the gradients of these linear relationships were the steepest for intestine and stomach. Based on the RNA measurements of liver and red and white muscle, Bulow (1987) concluded that the RNA/DNA ratio of white muscle was probably the "best" index of whole-body growth rate. Until recently, few other tissues had been examined for their relationship between RNA content and nutritional regime. The present data indicate that intestine and stomach RNA measurements are more sensitive than white muscle to alterations in nutrition (Fig. 2–4). However, growth rate consistently accounts for a greater proportion of the variability in the white muscle RNA data compared with the stomach and intestine (i.e. higher r^2 values). In the future, it may therefore be more sensible to use RNA measurements from several tissues for estimating the growth rate or nutritional status of fish.

The general decreases in tissue RNA content, RNA/protein ratio, and RNA/DNA ratio of many tissues with starvation are indicative of lower protein synthesis rates in these tissues (Smith 1981; Loughna and Goldspink 1984; Houlihan et al. 1988, 1989; McMillan and Houlihan 1988). However, it is important to note that protein synthesis rates can be altered by both RNA concentration (number of ribosomes) and RNA activity (Foster et al. 1991, 1992c). Changes in RNA content therefore only give an estimate of tissue protein synthesis rates, or growth rate. Tissue-specific differences in RNA concentration/activity and the sensitivity of tissues to a meal (McMillan and Houlihan 1988) probably account for the tissue-specific responses observed in the present study (Fig. 2–4).

Overall, it appears that stomach and intestine RNA measurements were generally the most sensitive indices of long-term nutritional regime and refeeding following a period of starvation. This appears reasonable because the protein synthesis rates in these tissues may be affected by the presence or absence of food. Interestingly, unpublished data from our laboratory (Noble 1990) indicates that a 6-d fasting period in rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*) decreases stomach protein synthesis rates. Furthermore, the point at which stomach protein synthesis rates decrease coin-

cides with the time of stomach emptying. This indicates that mechanical stretch within the stomach/intestine may influence protein synthesis rates within these tissues, although the precise nature of the signals has yet to be determined.

Most tissue CCO specific activities (with the exception of gill) increased linearly with whole-body growth rate (Table 4) and refeeding (Fig. 5). On the whole, it appears that CCO specific activities of the ventricle, stomach, and intestine are the most sensitive indices of both long-term nutritional treatment and short-term responses to refeeding following a period of starvation.

It was logistically impossible to maintain all the treatment groups in 70-L aquaria; therefore, the daily-fed group (treatment I) was maintained in a 2-m-diameter circular tank. Several authors (Johnston and Moon 1980; Goolish and Adelman 1987) have reported that tissue CCO specific activity is unaffected by locomotory activity, so this inconsistency in the experimental design is probably acceptable.

"Best" Indicator of Growth Rate (Nutritional Status)?

The results of this study indicate that many of the measured parameters were positively related to increasing growth rate after 35 d of maintenance under a controlled nutritional regime. These results agree with other studies (Bulow 1970; Buckley 1979; Goolish and Adelman 1987; Houlihan et al. 1989; Mathers et al. 1992). However, this study also investigated the speed of response of reported growth indices to short-term alterations in nutrition.

Of the tissue-somatic index data, liver was sensitive to both long-term growth rate and rapid changes in nutritional level. The present study also indicates that stomach, intestine, and white muscle RNA measurements are consistently "good" indices of growth rate and sensitive to alterations in nutrition. At the same time, stomach and intestine CCO specific activities and liver-somatic index are also linearly related to growth rate, although the low gradients observed in the refeeding experiment argue against tissue CCO specific activities as sensitive indices of changes in growth rate or nutritional status. The final choice of which parameter to measure will probably depend upon the available analytical facilities.

Although this study gives credence to the use of various growth indices under constant laboratory conditions, the extrapolation of these conditions to estimating growth (nutritional status) in wild populations must be a tentative step, as other factors (such as water temperature) have marked effects on the tissue-somatic indices, RNA concentration, and CCO activity of juvenile cod (Foster et al. 1992a, 1992b, 1992c).

Whilst it is probably too simplistic to rely on the use of any one measurement as a robust index of growth rate, the present results suggest that the use of multiple growth correlates (e.g. liver-somatic index, stomach, intestine, and white muscle nucleic acid measurements, and aerobic enzyme activities) may result in more accurate estimates of growth rate.

Acknowledgments

Special thanks to Norrie C. Ramsay for help with the fish dissections. A.R.F. was in receipt of a NERC CASE award.

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Components Analysis of a Density-Dependent Catchability Coefficient in a Salmon Hook and Line Fishery

Thomas F. Shardlow

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Shardlow, T. F. 1993. Components analysis of a density-dependent catchability coefficient in a salmon hook and line fishery. Can. J. Fish. Aquat. Sci. 50: 513–520.

The relationship between the catch per unit of effort (*C/E*) of salmon anglers and salmon abundance was determined by using simultaneous estimates of both *C/E* and salmon density (*n*). Salmon in their natural marine environment were observed as they approached fishing gear. The components of catch, such as the number of fish approaching, attacking, and biting the lure per unit of fishing effort, were recorded. *C/E* was not linearly related to density because the estimate of the catchability coefficient *q* increased with abundance. This result contrasts with previous empirical studies of catchability in which *q* declined with fish abundance. However, because only historical catch records had been used to estimate catchability, the interactions between the fishing gear and fish were not available in past research. This study suggests that feeding facilitation among salmon may be the mechanism underlying increased catchability with abundance.

Le rapport entre la prise par unité d'effort (*P/E*) des pêcheurs à la ligne du saumon et l'abondance du saumon a été déterminé au moyen d'estimations simultanées du rapport *P/E* et de la densité des populations de saumons (*n*). On a observé comment se comportaient les saumons, dans leur milieu marin naturel, alors qu'ils s'approchaient des engins de pêche. Les constituants qui déterminent la prise tels que le nombre de poissons qui s'approchent, qui attaquent et qui mordent au leurre par unité d'effort de pêche, a été relevé. Il n'y a pas de corrélation linéaire entre *P/E* et la densité de population du fait que l'estimation du coefficient de vulnérabilité à la pêche, *q*, s'accroît avec l'abondance. Ce résultat est en contradiction avec ceux d'études empiriques antérieures sur la vulnérabilité à la pêche où *q* diminuait lorsque l'abondance des poissons s'accroissait. Cependant, puisque l'estimation de la vulnérabilité à la pêche n'était fondée que sur des relevés historiques de capture, on ne pouvait pas faire intervenir les observations sur le poisson face aux engins de pêche dans les études antérieures. Cette étude donne à penser que la facilitation de l'alimentation du saumon peut constituer le mécanisme conduisant à une vulnérabilité accrue à la pêche avec l'abondance.

Received January 20, 1992
Accepted September 9, 1992
(J8497)

Reçu le 20 janvier 1992
Accepté le 9 septembre 1992

Catch per unit of effort (*C/E*) is often seen as an index of relative fish abundance. The application of this index for the purpose of measuring relative density or abundance of fish stocks had met, up to the mid-1960's, with little success. Large amounts of unexplained variability in estimates of average *C/E* made predictions of relative abundance unreliable (Paloheimo and Dickie 1964). Central to this problem, then and now, is an understanding of how *q*, the proportion of the population that is caught per nominal unit of effort, is related to abundance (*N*). In the simplest formulation, *q*, the catchability coeficient, is a constant proportion of *N*:

$$(1) \quad C/E = qN$$

where *C/E* is the catch per nominal unit of fishing effort.

Mechanisms that could theoretically give rise to variability in *q* have been discussed by Gulland (1964) and Paloheimo and Dickie (1964). Gulland (1964) considered changes in *q* with fish abundance as the most important mechanism because this type of variation in *q* "will change the whole shape of the relation between stock abundance and *C/E* from a proportional one to some type of curve." Paloheimo and Dickie (1964) proposed a model based on the theoretical interaction between randomly distributed schools of fish and fishermen searching for schools. Their prediction was that *q* is not constant but varies inversely with *N*.

Later investigations of how *q* varies with *N*, such as those by MacCall (1976), Garrod (1977), and Winters and Wheeler (1985), were based on comparisons of long-term catch and effort records with population size estimates gained from cohort analysis. These empirical estimates of *q* produced results consistent with the predictions of Paloheimo and Dickie (1964) in that *q* was shown to vary inversely with *N*. However, these studies, because they were concerned with models dealing with a whole fleet and estimates of whole cohorts, could not make direct observations of fish interacting with fishing gear.

Direct observation is important for several reasons. First, estimates of *q* gained through cohort analysis depend critically on assumptions about natural mortality rates (Gulland 1977; Ricker 1977). However, since age-specific natural mortality is never known with any certainty, a range of possible mortality rates is assumed and estimated *q* will vary depending on these assumptions about natural mortality (Ricker 1977). Second, the use of direct on-site measures of catch and abundance avoids the problems associated with long-term records that may show trends in *q* that are independent of density. Gulland (1964) pointed out that direct improvements in fishing technology continually increase the efficiency of a unit of effort. Therefore, for long-term records, the units of effort are not directly comparable. Most previous studies of *q* have used records spanning many years.

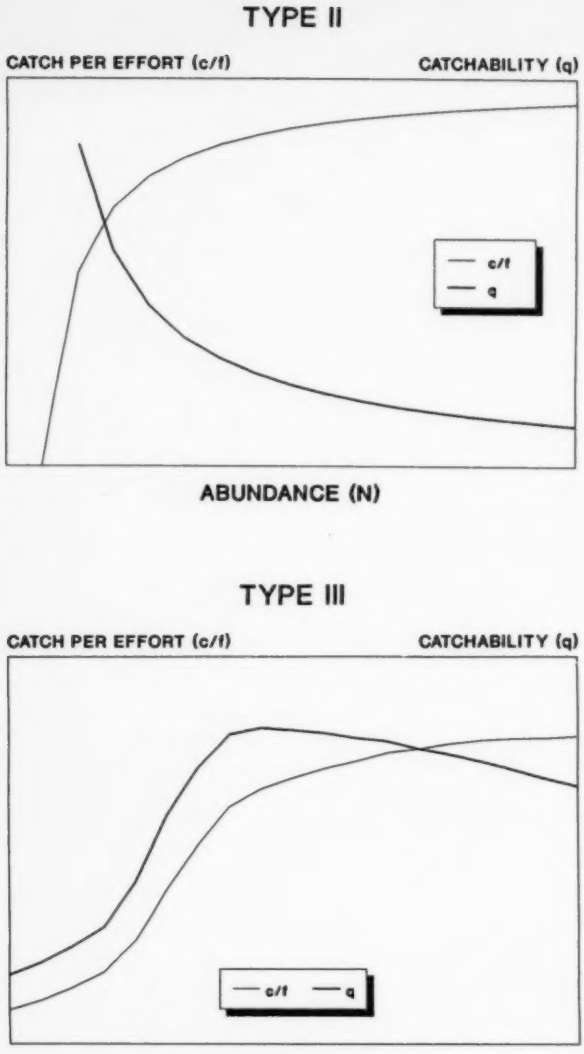

FIG. 1. Hollings (1959) type II and type III functional responses. In type II, q declines with N, while in type III, q increases with N.

In recent catchability studies, abundance estimates have been gained using more direct and fishery-independent methods. In addition, no time trends in q were involved.

Peterman and Steer (1981) related predator–prey theory to a salmon fishery using Holling's (1959) functional response types II and III (Fig. 1). The problems involved in estimating abundance from cohort analysis were avoided by summing localized catch and escapement records to gain an estimate of N. Their results were analogous to a type II response (Fig. 1). Bannerot and Austin (1983) and Crecco and Savoy (1985) also showed no time trends in q and used fishery-independent relative abundance estimates. More recently, Richards and Schnute (1986) and Richards (1987) used test angling CPUE and SCUBA measures of N in a rockfish hook and line fishery to compare general models of catchability. The results of these investigations again supported previous evidence that q declines with N.

Finally, by using catch records that compare annual or seasonal C/E with annual or seasonal records of N, much of the interaction of fishing effort with fish abundance is lost. Fishermen will encounter a wide range of densities or local abundance during the course of their operations over relatively short periods of time. The components of the behaviour of fish and the performance of fishing gear with variations in local abundance are the factors that influence catch success, and therefore q, most directly. The details of how these short-term differences in density and behaviour can contribute to variations in q are not available in existing catch records.

The purpose of this study is to determine the relationship between C/E and N for a fishery using a simultaneous measure of density and the components of catch.

Methods

The fishery investigated was the Strait of Georgia salmon sport fishery. The most common fishing method used in this fishery is trolling in which a lure is towed through the water from small boats operating near shore. This fishery has its counterpart in commercial salmon trolling where many lures are trolled at the same time from larger vessels. Some of the mechanisms that affect catch success of sport trolling, therefore, will also influence catch success in commercial hook and line fisheries.

The basic data requirements for an estimate of the relationship between C/E and N involve (1) a precise definition and measure of one unit of effort (E), (2) a record of catch during the unit of effort (C), and (3) an assessment of abundance (N) that does not rely on C/E for a measurement of N, that is, a fishery-independent measure of N that is coupled closely in time with measures of catch success.

In this study, effort is defined as the amount of time a single hook and line unit of sport gear was trolled through the water.

Observing and Recording Catch Information

A towed underwater video camera was developed to record the behaviour of salmon under ambient conditions as they responded to trolled sportfishing gear. The system included a clear acrylic stabilizer unit that housed the camera unit while being towed. A 90° lens and auto-iris facilitated video recording.

Attacks by individual salmon were categorized into various types of behaviours. These behaviours ranged from apparent disinterest of fish that swam through the field of view and did not orient toward the lure to direct pursuit and biting of the lure.

The basic behaviour classifications used in the analysis were encounters, close encounters, strikes, and hooked (Table 1). An encounter involved a behaviour in which a salmon oriented and swam toward the lure. Often this pursuit was short lived and the fish would swim away without coming in close contact with the lure. Close encounters included only those fish that came within close proximity of the lure (within 1 m). Strikes were recorded when a fish took the lure in its mouth. Finally, the hooked category involved cases where fish were caught by the hook and were retrieved using a rod and reel.

Density Estimation

A hydroacoustical estimate of salmon abundance was gained through the use of a chart recording echo sounder. This system consisted of a Furuno fm21d echo sounder with a frequency of

TABLE 1. Encounter responses, fish strikes, fish hooked (numbers), and salmon density by sampling location.

Location	Encounters		Fish strikes	Fish hooked	Salmon density (fish/240 000 m^3)
	Total	Close			
Sutil Point	14	14	0	0	385
	17	17	1	1	889
	0	0	0	0	417
	5	5	0	0	435
Gowers Point	5	4	2	0	176
	5	4	1	1	292
	6	6	1	0	268
	13	5	0	0	374
	10	7	0	0	298
	21	8	0	0	596
	48	21	4	0	760
	17	6	1	0	467
Active Pass	2	2	1	1	50
	6	4	0	0	63
	22	7	1	1	280
	40	18	1	0	427
	9	6	0	0	168
	21	13	3	2	232
	28	11	1	1	332
	7	3	1	0	180
	46	7	1	0	328
	84	20	2	1	427
Cowichan Bay	46	3	1	0	588
	29	3	0	0	866
	14	0	0	0	595
	8	2	1	1	383
	2	1	0	0	523
	0	0	0	0	571
	0	0	0	0	216
	1	1	0	0	68
	7	0	0	0	433
Cape Mudge	8	5	0	0	48
	2	0	0	0	72
	10	8	7	2	100
	6	1	1	0	20
	4	1	1	1	68
	16	6	6	2	52
	7	3	0	0	44
	5	5	2	0	136
	0	0	0	0	4
	7	4	3	0	16
	3	2	1	1	32
	5	3	3	1	4
	10	1	0	0	108

50 kHz. The towed transducer produced a beam angle of 42° and the acoustic impulse was modified with a time-varied gain. The gain modification corrected for depth variations in target strengths. This system is designed to count individual fish targets.

A common problem in hydroacoustical estimates of N is the species identification of echo targets. Often, gillnetting or other techniques are employed to determine proportionate species composition. In this study, such techniques were not used, since individual fish could often be seen on the television monitor and the echogram at the same time. From these simultaneous observations, echo targets could be identified as spiny dogfish (*Squalus acanthias*), Pacific herring (*Clupea pallasi*), and Pacific salmon (*Oncorhynchus* spp.).

Hydroacoustical transects were divided into 30-min intervals. This volume was further stratified into 10-fathom (18.2-m) depths within which the video assembly was centered. The basic density unit used in the analysis was the number of salmon targets (corrected for volume sonified) counted within this stratified volume. The basic effort unit was the 30 min during which a single hook and line unit of gear was trolled through the water.

Five locations within the Strait of Gerogia were selected for continued monitoring on the basis that they were free from submerged hazards and strong winds or currents: Gower Point, Cape Mudge, Sutil Point, Active Pass, and Cowichan Bay.

Sampling occurred from May through October. A survey of sportfishing methods and catch success was also conducted at these locations. The results of the survey were used to set fishing patterns and gear in order to mimic local fishing practices.

Results

Salmon Behaviour

Although the ways in which salmon approached the fishing gear were variable, there are some generalizations that can be described. Only on one occasion did salmon that were seen in the field of view of the camera not pursue the lure. This is not to say that all salmon that saw the lure pursued it, but rather that those salmon close enough to be seen through the camera were already in pursuit. The number of salmon approaching the lure at any one time varied from one to four. Some approaches toward the lure lasted only a second or two, while other fish followed the lure for up to 10 min.

The salmon observed invariably approached from a position behind the lure; however, the intensity of pursuit was varied. In the most intense pursuits, it seemed the decision to take the lure was made at some distance outside the camera's field of view. In these cases, the fish made no apparent change in direction or velocity from the time it was first seen to the time it struck the lure. These approaches were very rapid and often resulted in a hooked fish. In other instances, the salmon would approach at slower speeds and make several close passes by the lure, turning away and returning frequently. Some of these differences in approach can be ascribed to species differences. Pink salmon (*Oncorhynchus gorbuscha*) characteristically approached to a position very close (several centimetres) directly behind the lure and tracked the lure's path very closely. This pursuit was ended either by a bite, which was a simple opening and closing of the fish's mouth about the lure, or by swimming away. In contrast, coho salmon (*Oncorhynchus kisutch*) approached the lure quickly. However, instead of closely following the path of the lure, they veered off to one side just as they came close to the lure and struck at the lure from the side or top. This difference in attack behaviour can likely be attributed to the different prey items taken by these species. Coho are more piscivorous than pink, the latter's diet being composed mainly of zooplankton.

Test Fishing versus Sport Fishing

Samples of the anglers' catch and effort were taken at the same locations and during the same times as test fishing. Ten pairs were analyzed for differences in C/E. No significant statistical difference existed between the paired comparisons ($t = -0.23, p = 0.58$). In fact, angler and test fishing values are very similar (test mean $C/E = 0.47$, angler mean $C/E = 0.44$). This suggests that test fishing samples can be used to represent the normal sportfishing dependence of catch success on salmon abundance.

Observations of catch components and abundance are listed by location in Table 1. The number of total encounters and close encounters per 0.5 h are listed as well as the number of fish striking and finally being hooked during the same time period. The salmon density (fish targets per volume sonified) found during each 0.5-h fishing stratum is also listed.

Visibility

Differences in turbidity or water clarity were observed between locations. In some locations, it was evident from reviewing video tapes that the observer could see fish at a greater distance from the camera than could be seen at more turbid locations. This, coupled with the observation that salmon approached to variable distances behind the camera, means that in locations where visibility for the observer was high, fish could be seen approaching the lure that would be observed when visibility was low. Because no direct measure of visibility was made, standardizing samples for visibility differences is difficult. For this reason, a procedure was adopted whereby fish that approached to within very close proximity of the lure (within 0.6 m) were taken to represent close encounters. For comparisons between locations, only close encounters (Table 1) were used.

Comparisons between Locations

In Fig. 2, close encounters are compared with density for two locations. The remaining locations have either a low probability associated with the regression or have variances too large to show differences between locations. Gowers Point and Active Pass show a difference in slopes of encounters against density using a t-test comparison for regression coefficients (Zar 1974) ($t = 1.78, p < 0.10$) (Fig. 2).

Nonlinearity

Within some of the five locations, a second-order polynomial regression ($y = ax + bx^2$) indicated a strong nonlinear encounter response over the densities sampled (Fig. 3 and 4). These nonlinear regressions are based on total encounters (close plus distant) per standardized unit of effort. For both Gower Point (Fig. 3) and Active Pass (Fig. 4), a linear relationship between encounters and density can be rejected ($p < 0.05$). This conclusion is made in consideration of the extreme assumptions that model error was entirely attributable to either density measurements or the number of encounters (e.g. see Schnute et al. 1990). For either assumption, likelihood ratio tests (Schnute and Groot 1992) indicate that b (see equation (4)) was significantly ($p < 0.05$) different from zero for both Gower Point and Active Pass. In Cowichan Bay, however, encounters were linearly related to fish abundance ($p = 0.54$). The remaining two locations either have points over a limited range of densities or have too few samples to show a trend in encounter rates.

Discussion

Previous empirical studies have indicated that q varies inversely with N. The results of the present study suggest the opposite, that is, q increases with N. The inverse relationship between q and N has been attributed to the effect of gear saturation (Rothschild 1977) when, for example, a net or trap becomes full of fish and increases in N cannot result in a higher catch rate. In the case of hook and line fishing, saturation would occur when the hooks are occupied by fish at a rate faster than the fish can be removed. Thus, as N increases, the proportion of N caught per unit of effort decreases. However, in the sport fishery investigated, fish abundances never reached a level where the catch of one fish impeded the catch of a second. The highest recorded catch per line-hour was one at Cape Mudge. It takes approximately 5 min to remove a fish from the line and resume fishing. Other fisheries as well, such as the commercial troll fishery, may seldom experience salmon densities high enough to reduce catchability during a fishing season. In fact,

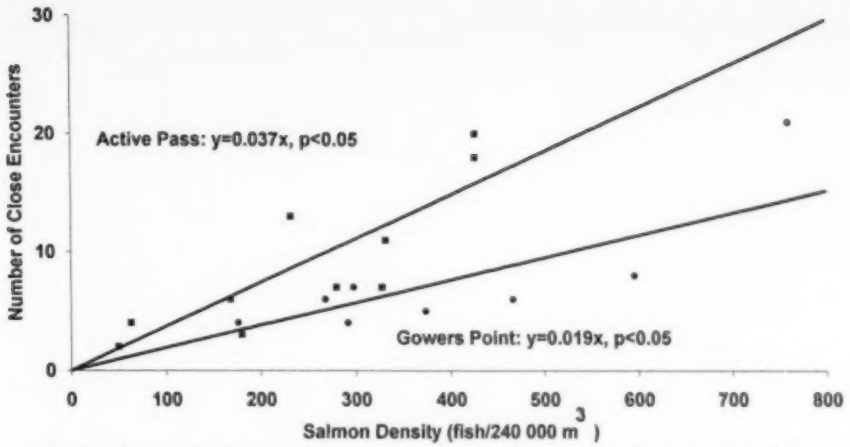

FIG. 2. Regression of close encounters and salmon density at Active Pass and Gowers Point.

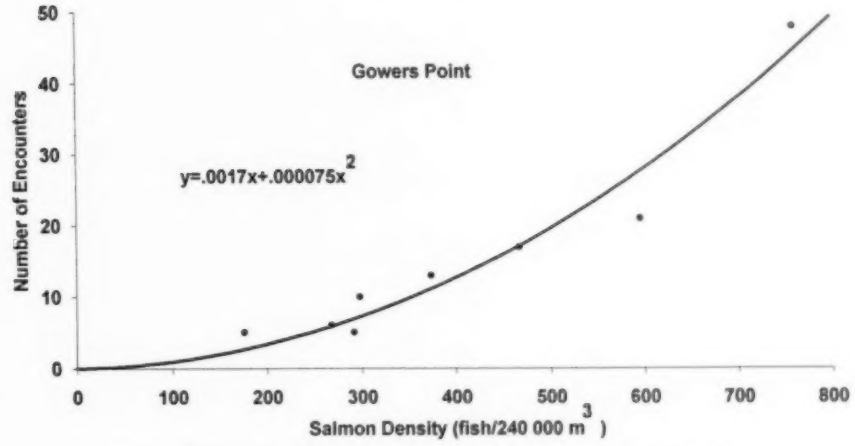

FIG. 3. Regression of total encounters and salmon density at Gowers Point.

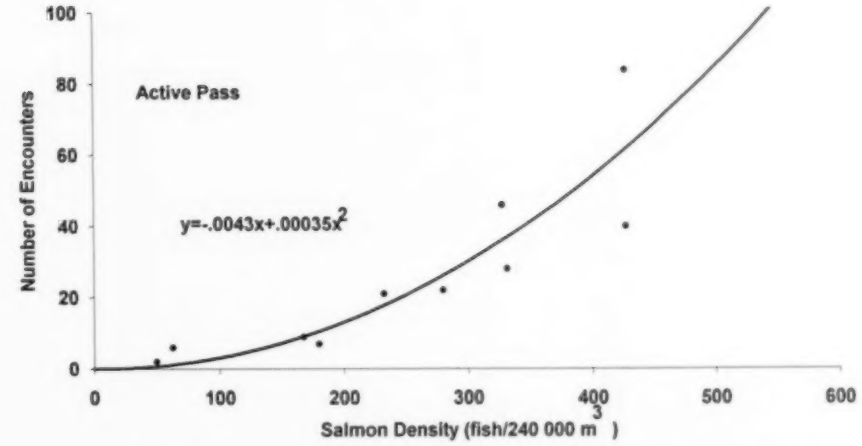

FIG. 4. Regression of total encounters and salmon density at Active Pass.

the opposite can be true. Catchability may increase with N over the range of N mostly encountered by hook and line fisheries.

Relationship between q and n

The relationship under discussion in this study thus far has been between the number of approaches or attacks to the line per unit effort (a/E) and local density n (where n = some subset of N, the total population):

$$(2) \quad a/E = Pn$$

where P is the fraction of the population n that is attracted to the gear. To find C/E, one needs to know the proportion of the attracted fish that are caught (p_0). Therefore:

$$(3) \quad C/E = Pp_0n$$

and catchability $q = Pp_0$. For the present, p_0 will be some constant value.

From the results shown in Fig. 3 and 4, P is not constant and the rate of attracting fish increases with abundance. In effect, this means that the efficiency of the lure to attract fish increases with abundance. Efficiency can depend on the volume swept or "searched" by the fishing gear. The problem now becomes to determine how a larger volume is searched at high fish density than at low density.

The concept of predators providing important signals that facilitate prey detection by conspecifics is well documented (see Curio 1976 for summary). Behavioural interactions between feeding fish have been commonly observed both informally by fishermen and experimentally by scientists. Almost any angler can recount observing several fish chasing his lure at the same time with one fish leading the chase and the other fish following behind. The suggestion here is that the attack on the lure by one fish prompted other fish to attack. Commercial salmon trollers, believing that one hooked sockeye salmon (*Oncorhynchus nerka*) on the line will attract others, will delay retrieving hooked fish from the water. This type of attack or feeding facilitation has also been observed experimentally. Protasov (1970) cited the work of Voronin (1957) as having experimentally established "that the visual link between fish of the same species during feeding is based on conditional imitative reflexes." That is, individuals in a conspecific group will imitate the feeding behaviour of nearby fish. Experiments conducted by Voronin (1957, cited in Protasov 1970) on Black Sea bass, pickerel, perch, and roach and studies by Markl (1972) on piranhas show that this phenomenon is widely seen in fishes. It is reasonable to expect that salmon feeding in the ocean will exhibit the same "imitative food-getting reflex." This facilitation can be interpreted as the transferring of information about the availability of food (the lure) from salmon near the lure to salmon at farther distances from the lure. This phenomenon, in effect, increases the volume swept by the lure as more fish are available to transfer information about the presence of food.

In equation (2), P is not a constant proportion of n, but is made up of two components: a_1, the initial proportion attracted to the lure, and a_2, the additional component of n attracted by the feeding response of a_1. Thus:

$$a/E = a_1n + a_2n(a_1n).$$

Let $a_2a_1 = b$:

$$(4) \quad a/E = a_1n + bn^2.$$

This quadratic expression forms the biological model that was applied to the data in the form of a second-order polynomial regression of encounters against density. Figures 3 and 4 show that the second term (b) is significant and positive ($p < 0.05$) for equation (4) for both Gower Point and Active Pass.

p_0 was defined as the proportion of the fish attracted that were actually caught. However, p_0 can be further decomposed to $p_0 = p_1 \cdot p_2$ where p_1 is the proportion of strikes and p_2 is the proportion of those fish that struck that were caught.

This separation of p_0 into two components has the advantage of allowing for the separation of the biological and mechanical aspects in the catch component. The strikes per encounter p_1 is associated with biological variables such as hunger and the stimuli presented by different lure types whereas p_2 can be thought of as being associated with mechanical variables, such as probability of the hook being set once it is struck by a fish. Factors such as hook size, sharpness, and position relative to the lure and the fish's mouth are important variables in this case. In this study the ratio of strikes to close encounters was $p_1 = 0.17 \pm 0.04$ and the fraction hooked out of strikes was $p_2 = 0.31 \pm 0.06$; q now has two additional components to those given in equation (3) where

$$q = (a_1 + bn)p_0.$$

Now $p_0 = p_1 \cdot p_2$ and

$$(5) \quad q = (a_1 + bn)p_1p_2).$$

This q, however, is an estimate of catchability only for a very small volume of the Strait of Georgia. Although it is beyond the scope of this work, the final consideration in determining q for a whole fishery is to know the relationship between n and N. In this study, n is the population of salmon in the standard sample volume of ocean and N is the abundance of salmon in the total population. Stratification of samples was conducted such that the number of approaches to the lure, and ultimately catch per unit efort, could be predicted over varying densities. It was assumed that the volume selected for n represented a volume within which the population of salmon was relatively available to the fishing gear. Larger vertical sample volumes showed no relationshp between the number of salmon encounters and salmon abundance. Similar difficulties would arise when relating the parameters of catch and density found within a sampling location to the larger volume of the Georgia Strait.

This study shows q to increase with density, while all the others referenced show q to decrease with density. As discussed earlier, the Paloheimo and Dickie (1964) model was based on the interaction between fishermen searching for schools and changes in the number and radius of schools. These authors did assume, however, that once a school was located, a fishing vessel would catch a constant proportion of that school. Therefore, as Paloheimo and Dickie (1964) pointed out, the catchability coefficient was assumed to be a constant proportion of the density or the abundance in a school. It was beyond the scope of my study to consider the relationship between the size and number of salmon schools in the Strait of Georgia and angler fishing success. My findings can, however, be interpreted as being the relationship between catch per unit of nominal effort and changes in density within a school or aggregation of salmon. This relationship would be more pronounced in pink and sockeye which tend to school more than other salmon. In this respect, I have argued that q is not a constant but will increase with local density.

The effect of this latter type of density-dependent catchability coefficient would be most pronounced when overall density, defined as the number of schools by Paloheimo and Dickie (1964), is low, but the densities encountered during fishing

operations remain constant. An example of this situation occurs when overall abundance is low and salmon are aggregated at the popular fishing locations. At high overall abundance, however, when the number of schools are numerous and the capacity of the fishing vessel to respond by increasing area searched is limited, then theoretically an asymptote in catch per effort will be reached (Paloheimo and Dickie 1964). These authors discussed limitations in area searched and proposed several mechanisms that could produce an asymptote at high levels of overall density.

The effect, then, of having an increasing catchability coefficient at low density and a decreasing catchability coefficient at high densities is a sigmoid-shaped function for catch per unit of effort over density. Little is known about the nature of spatial heterogeneity of salmon in the Strait of Georgia that could be related to this question. This type of sigmoid curve must thus remain as speculation at this point. However, Peterman and Steer (1981) have discussed the application of Holling's (1959) type II and type III functional responses (Fig. 1) to salmon sport fisheries. The type III response is a sigmoid function, while the type II response is analogous to Paloheimo and Dickie's (1964) relationship for catch per unit of effort to density. Peterman and Steer (1981) concluded that a type II and not a type III response best described their sport fishery. The fitting of a type II response by Peterman and Steer (1981), however, could have resulted as an artifact of large errors in the estimation of abundance (Shardlow and Hilborn 1985). Moreover, as Shardlow and Hilborn (1985) pointed out, the mechanism proposed by Peterman and Steer (1981) assumed that salmon density, at locations where fishermen could fish effectively, would remain constant regardless of annual changes in salmon abundance. In the fishing locations sampled in the current study, estimated density varied 10-fold (Table 1). It seems likely that similar density fluctuations could occur on the temporal and spatial scales measured by Peterman and Steer (1981).

In the present study the results are more analogous to a type III than a type II functional response because type III responses occur when the rate of effective search, for example, area searched by a predator, increases with prey density (Hassel 1978). It is argued in this current study that the search component or volume searched increased with density through the facilitation effect in salmon predatory behaviour. This could also be compared with the cooperative effect discussed by Rothschild (1977), where vessels instead of fish cooperate in search of prey.

Finally, all functional responses eventually reach a point where increases in prey density do not result in any further increase in rate of prey capture. This plateau or maximum rate of prey capture can be reached when, as discussed by Holling (1965), the time required to handle and process prey consumes all the time available for predatory activity. Although such a plateau was clearly not found during this study, a plateau would be expected to occur when all the time spent fishing was taken up in hooking and retrieving fish. Fowler (1980) documented this effect for whaling operations when the components of effort such as time spent in searching for, handling, and transporting the catch were studied. Alternatively, a plateau could also be reached when additional fish attracted to the lure per unit time, a/E, could not be hooked because the lure was already occupied by a fish. This type of hook saturation was discussed by Rothschild (1977).

The average rate of catch experienced during this study was 0.47 and 0.44 fish per lure hour for test fishing and anglers, respectively. The density at which an asymptote would be reached (n_{max}) can be calculated from equations (3) and (5) using the parameter estimates gained from Active Pass and Gowers Point in the quadratic formula, as follows:

$$n_{max} = \frac{-a_1 \pm \sqrt{a_1^2 + \dfrac{4bC/E}{p_1 p_2}}}{2b}.$$

n_{max} is found to be approximately three times higher than the highest density found in this study. The reliability of any estimate of q for an entire fishery such as the Strait of Georgia is only as good as the understanding of how n, the population of salmon encountered while fishing, relates to N, the total population. Further work in this area would be required before specific quantitative predictions about catch success in relation to abundance could be made for a specific hook and line fishery.

Acknowledgements

W. E. Neill provided the opportunity for this work. Discussions with N. J. Wilimovsky, B. E. Riddell, and particularly R. Hilborn improved the document substantially. I thank B. Gazey, J. Schnute, and B. Smith who assisted in statistical problems and G. Bodgero, D. Peacock, and C. Murray who assisted in field work.

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Mercury in Macroinvertebrates from Swedish Forest Lakes: Influence of Lake Type, Habitat, Life Cycle, and Food Quality

Helena Parkman

Swedish Environmental Research Institute (IVL), Box 21060, S-100 31 Stockholm, Sweden

and Markus Meili

Institute of Limnology, Uppsala University, Box 557, S-751 22 Uppsala, Sweden

Parkman, H., and M. Meili. 1993. Mercury in macroinvertebrates from Swedish forest lakes: influence of lake type, habitat, life cycle, and food quality. Can. J. Fish. Aquat. Sci. 50: 521–534.

Mercury concentrations in lacustrine macroinvertebrates were concurrently studied in eight remote Swedish forest lakes of differing dystrophy, acidity, and eutrophy. The aim was to assess the influence of ecological factors (taxon, habitat, and feeding habits), chemical factors (characterizing different types of lakes), and regional and climatic factors (Hg deposition and mean temperature) on the accumulation of Hg. Concentrations varied from <50 to >5000 ng Hg·g dry weight^{-1}. A large part of this high variability could be ascribed to differences in water and sediment chemistry, ecological niches, and species-specific seasonalities. Both taxonomic composition and Hg concentrations were highly dependent on the type of lake. Concentrations were highest in acidic dystrophic lakes and lowest in oligotrophic lakes. Mean Hg concentrations in the examined taxa within a lake differed 100-fold. Contrary to widely held views on biomagnification, the lowest concentrations among profundal chironomids were found in predators whereas the highest concentrations occurred in detritivores. Seasonal variations were negligible in some taxa but considerable in others and appeared in some taxa to be related to the life cycle. We conclude that Hg accumulation in macroinvertebrates is largely determined by feeding behaviour and food quality.

La concentration de mercure trouvé dans les tissus de macro-invertébrés lacustres a été mesurée simultanément chez des sujets de huit lacs isolés dans des forêts suédoises et à différents niveaux de dystrophie, d'acidité et d'eutrophie. L'objectif était d'évaluer l'influence de facteurs écologiques (taxon, habitat et habitudes alimentaires), de facteurs chimiques (qui caractérisent différents types de lacs) et de facteurs climatiques et régionaux (dépôt de Hg et température moyenne) sur l'accumulation du mercure. La concentration variait entre <50 et >5 000 ng Hg·g en poids sec^{-1}. Cette forte variabilité pouvait être largement attribuable à des différences dans la chimie de l'eau et des sédiments, à l'existence de niches écologiques différentes et à des variations saisonnières caractéristiques d'espèces données. Tant la composition taxonomique que la concentration du Hg dépendaient fortement de la nature du lac. La concentration était la plus élevée dans les lacs dystrophes acides et la plus faible dans les lacs oligotrophes. Chez les taxons examinés, la concentration moyenne dans les tissus à un lac donné variait par un facteur de 100. Contrairement à l'avis couramment reçu sur la bio-amplification, la plus faible concentration de Hg chez les chironomidés situés en profondeur a été observée chez des prédateurs alors que la concentration la plus élevée l'a été chez des détritivores. Chez certains taxons, les variations saisonnières étaient négligeables, mais elles étaient considérables chez d'autres, et dans certains, elles semblaient liées au cycle biologique. Nous parvenons à la conclusion que l'accumulation de Hg dans les tissus des macro-invertébrés est largement déterminée par la qualité des aliments et le mode d'alimentation.

Received December 10, 1991
Accepted September 3, 1992
(JB323)

Reçu le 10 decembre 1991
Accepté le 3 septembre 1992

In about 10 000 Swedish lakes, mercury concentrations in fish are estimated to exceed the health advisory limit for human consumption (1 mg·kg wet weight^{-1} in the flesh of 1-kg northern pike (*Esox lucius*)) (Håkanson et al. 1990a). Most of these lakes are situated in remote forest areas that have never been affected by any known direct discharge of Hg. However, Hg is efficiently accumulated in lacustrine animals (e.g. Jernelöv et al. 1975; Huckabee et al. 1979; Meili 1991a). In fish (e.g. Meili 1991a), and probably also in invertebrates (Lock 1975), uptake of Hg occurs primarily from the food. As a result, the concentration in animals is largely controlled by the concentration in their prey and ultimately by the degree of biomagnification in food chains (e.g. Jernelöv and Lann 1971; Jernelöv et al. 1975; Meili 1991a, 1991b).

Both littoral invertebrates and profundal detritivorous chironomids serve as a major food source for fish in most lakes. They may play a quantitatively important role in the remobilization of sediment Hg and in regulating the fluxes of bioavailable Hg in lakes. In boreal forest lakes, the total input of Hg is usually dominated by the inflow from the watershed (Meili 1991c). Humic matter carrying this Hg (Johansson et al. 1991; Mierle and Ingram 1991) often dominates the organic fluxes in the lakes (Meili 1992) and also constitutes the dominant fraction in sediment organic matter. Particulate and dissolved humic matter can be utilized by filter- or deposit-feeding invertebrates, either directly or via bacteria and bacterivores (e.g. Tranvik 1989; Hessen et al. 1990). These detrital food chains may be crucial for the availability and enrichment of Hg

TABLE 1. Lake characteristics, water and sediment chemistry, and Hg concentrations in eight Swedish forest lakes sampled in 1985–86. The values for pH and water colour are the ranges found in the water column including the depths of profundal sampling whereas the range of P concentrations represents the productive surface water. Locations: C = central Sweden, S = southern Sweden.

Lake					Watershed	Water column			Sediment at profundal sampling				1-kg
Name	Location	Type[a]	Area (km^2)	Residence time (yr)	area (km^2)	pH	Colour (mg $Pt \cdot L^{-1}$)	Total P ($\mu g \cdot L^{-1}$)	Depth (m)	C (% dw)	C/N ($g \cdot g^{-1}$)	Hg ($ng \cdot g\ dw^{-1}$)	northern pike mean Hg ($mg \cdot kg\ ww^{-1}$)
Bottentjärn (BO)	C	I	0.15	0.4	4.2	5.0–6.4	70–130	8–12	4–4.5	13–16	12–15	130–245	2.00
Skärhultssjön (SK)	S	I	0.36	0.8	6.0	5.2–6.1	80–190	10–21	4–6	19–21	13–14	210–300	1.10
Blacksåstjärn (BL)	C	II	0.12	0.5	2.2	6.0–7.1	75–130	11–14	3–6	12–16	11–13	120–220	1.57
Löjesjön (LÖ)	S	II	0.31	0.8	2.1	6.1–6.6	75–125	18–23	1.5–2.5	23–25	13–14	180–250	0.63
Loppesjön (LO)	C	III	0.28	1.2	4.4	6.3–7.6	45–80	15–24	6–8	7–10	10–11	90–140	0.61
Gårdsjön (GÅ)	S	III	0.15	1.9	0.9	6.1–7.3	20–60	9–20	4–5	26–29	12–14	210–280	0.58
Stensjön (ST)	C	IV	0.59	2.5	3.6	5.8–6.6	40–80	7–13	5–8	12–17	14–16	175–225	0.61
Holmeshultasjön (HO)	S	IV	0.69	1.8	6.0	6.3–7.3	20–65	9–15	3–6	14–19	10–12	210–300	0.57

[a]Lake types are characterized as follows:

	I. Acidic dystrophic	II. Neutral dystrophic	III. Mesotrophic	IV. Oligotrophic
Humic status	Meso- to polyhumic	Meso- to polyhumic	Oligo- to mesohumic	Oligo- to mesohumic
Trophic status	Oligotrophic	Oligotrophic	Mesotrophic	Oligotrophic
Acid status	Acidic	Slightly acidic to circumneutral	Circumneutral	Slightly acidic to circumneutral

in food webs, since bacteria both accumulate (Ramamoorthy et al. 1977) and transform mercurials (reviewed by d'Itri 1990; Winfrey and Rudd 1990), and may be of particular importance in benthic habitats. Consequently, zoobenthos probably constitutes a major transport route for sediment Hg into the fish community and indirectly to piscivores such as northern pike or man. Moreover, emerging insects may contribute to the transport of sediment Hg out of the system. Bissonnette (1975) estimated that an annual average of 0.08–0.37% of the total Hg content in surface sediment (0–1 cm, four lakes) was transported via chironomids into predatory fish, while emergence of adult insects accounted for a Hg transport from one of the lakes of about 0.04%. These fluxes are small when compared with the Hg pool in the sediment but significant when related to the waterborne Hg pool, which in remote Swedish forest lakes is only about 1% of the pool in surface sediment (0–1 cm), given a sediment water content of 90% (cf. Meili et al. 1991).

Most macroinvertebrates belong to benthic communities, which potentially results in a high exposure to Hg. Hg concentrations in sediments are many-fold higher than in the overlaying water on a volumetric basis, owing to the accumulation of Hg-carrying particulate matter. Moreover, the cycling and speciation of Hg are heavily affected by redox conditions that often dramatically change at the sediment surface (e.g. Korthals and Winfrey 1987; Winfrey and Rudd 1990). Consequently, Hg uptake by sediment-dwelling profundal animals is presumably favoured by the combination of high Hg concentrations in their diet as well as in their physical environment, low oxygen concentrations, and higher ingestion rates necessitated by the low food quality of detrital organic matter.

Hg concentrations in littoral and profundal macroinvertebrates have been analyzed in a number of contaminated or perturbed systems (for reviews, see Huckabee et al. 1979; Särkkä 1979; Surma-Aho et al. 1986). From undisturbed freshwaters, however, little information is available about the Hg content of these animals (Johnels et al. 1967; Olsson, 1976; Huckabee et al. 1979; Göthberg 1983; Surma-Aho et al. 1986).

Lacustrine invertebrates comprise a large number of species and developmental stages with widely differing feeding habits, complex habitats, and over a 1000-fold range in individual weight. This diversity calls for detailed investigations to evaluate the role of invertebrates in the Hg accumulation by fish. Studies including the ecology of these animals can also provide valuable clues on the turnover of bioavailable Hg within lakes. However, where invertebrates are analyzed in comparative Hg studies, ecologically differing taxa are usually pooled into groups such as chironomids or even benthos as a whole.

In this study, Hg concentrations were analyzed in the macroinvertebrate community of different types of remote Swedish forest lakes. The lakes are situated in two regions differing in climate and atmospheric deposition. Macroinvertebrate Hg concentrations and their seasonal variations are compared and statistically analyzed in the light of regional and climatic factors such as Hg deposition and mean temperature, chemical factors characterizing different types of lakes, and ecological factors such as taxon, habitat, life cycle, and feeding habits. The study is part of a comprehensive project focusing on the turnover of Hg in remote Swedish forest lake ecosystems.

Study Lakes

Macroinvertebrates were collected in eight small forest lakes, which were selected as four pairs of different types to obtain a range representative of Swedish headwater lakes. The four lake

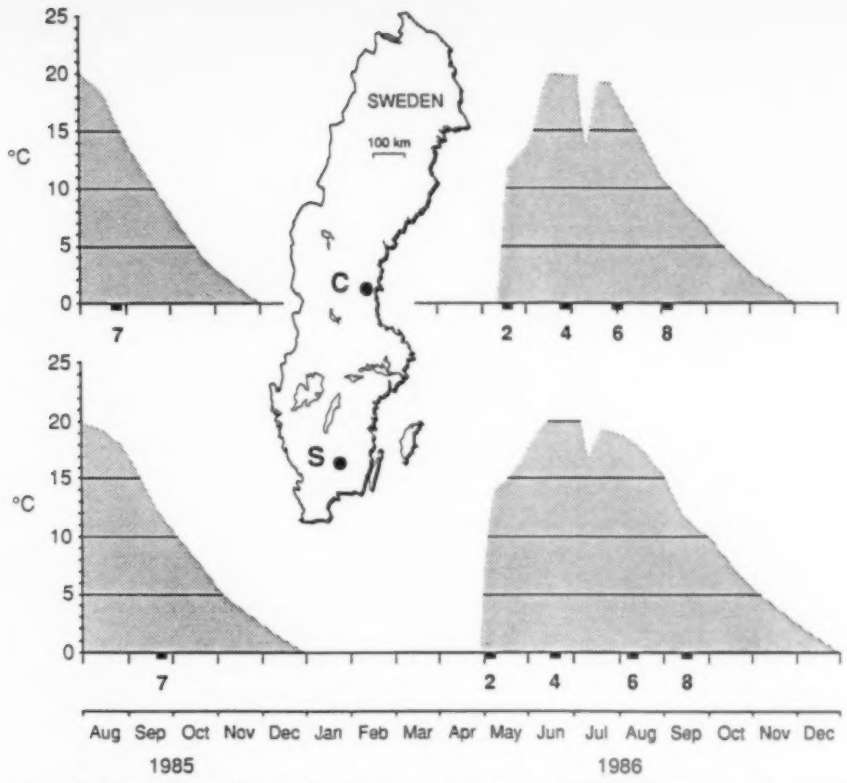

FIG. 1. Location of the study areas in central (C) and southern (S) Sweden and average surface water temperatures and sampling periods (2, 4, 6, 7, and 8) in each region, 1985–86.

types differ in morphology, humic content, acidity, trophic state, and fish communities and are henceforth referred to as acidic humic, neutral dystrophic, mesotrophic, and oligotrophic (Table 1). One lake within each pair is located in central Sweden (region C, Delsbo, Hudiksvall, Hälsingland, 62.6–7°N, 16.6–8°E), and one is located in southern Sweden (region S, Aneboda, Växjö, Småland, 57.1–2°N 14.4–7°E) (Fig. 1). Both regions are dominated by coniferous forest and mire, shallow podzolic soils, and igneous bedrock. Region C is situated about 500 km north of region S, has a lower mean temperature, and lakes are ice-covered for 1–2 mo longer than in region S. Hg concentrations in fish are higher in region C despite the lower impact from current atmospheric emissions of Hg (Håkanson et al. 1990a). More detailed data on the lakes and their catchment areas are given elsewhere (Meili 1992).

Methods

Macroinvertebrates were collected five times during the ice-free period of one year (August 1985 to September 1986). Sampling periods were chosen to follow the course of the biological year rather than calendar days. A year, starting at ice accretion, was therefore divided into 10 intervals with a similar number of degree-days >0°C in the warm strata of the water column. Sampling occasions were numbered referring to these intervals. The sampling intervals were adapted to the climate of each region so that water temperatures were similar in all lakes during a sampling period (Fig. 1). Period 7 was sampled in 1985 and periods 2, 4, 6, and 8 in 1986.

From littoral vegetation, animals were collected with a colander. From soft bottoms, sediment was sampled by dragging framed nylon net bags with an opening of 0.1 m^2 and a length of 1 m along the sediment surface. To obtain profundal animals from similar temperature and oxygen conditions the dragging was performed at a depth of 3–8 m or in the deepest area of shallower lakes (Table 1). Soft bottom fauna was isolated by screening the sediment through a 0.5-mm sieve. For defecation to take place, the animals were placed in lake water for about 8 h without food. The animals were sorted, counted, and, after removing excess water by placing the animals on filter paper for 1 min, weighed and frozen. For a rough quantification of the profundal fauna, four samples were taken in the same area with an Ekman grab (0.225 m^2) and preserved in 70% ethanol.

Sediment samples (0–1 cm) were collected with a gravity corer (Willner type, 65-mm diameter) in the middle of August (region C) and September (region S) 1986. Cores were taken from three depths: in shallow water (at about one third of maximum depth), at an intermediate depth, and in the deepest part of the lakes. Complementary samples from the same depths but different sediment layers (0–3 and 3–6 cm) were collected in region C in May 1988. Samples of water (Meili 1992), zooplankton (M. Meili, in prep.), and fish (Meili 1991a) were taken simultaneously with benthic sampling.

Frozen invertebrate samples were thawed and the animals separated into taxonomic groups (Table 2), mostly to the genus

TABLE 2. Examined taxa of macroinvertebrates and their ecology.

Taxon	Main habitat[a]	Diet (references)[b]
Crustaceans		
Isopoda		
Asellus aquaticus	L plants + sediment	Plants, plant detritus (1)
Insect larvae		
Megaloptera		
Sialidae		
Sialis lutaria	L + P plants + sediment	Chironomids, small animals (1, 2)
Odonata		
Zygoptera	L plants	Chironomids, zooplankton (3, 4)
Anisoptera		
Aeschnidae	L plants	Macroinvertebrates: chironomids, zygopterans, chaoborids, coleopterans (3, 5, 6)
Libellulidae	L plants + sediment	
Diptera		
Corethridae		
Chaoborus	P water + sediment	Zooplankton (7)
Chironomidae		
Procladius	P sediment (+ water?)	Chironomids, zooplankton (8, 9, 10)
Demicryptochironomus	P sediment (+ water?)	Oligochaetes, "predatory" (11)
Cryptochironomus	P sediment	Oligochaetes, "predatory" (12, 13)
Sergentia	P sediment	"Mud particles" (14)
Stictochironomus	P sediment	Detritus (9, 15)
Chironomus spp.	P sediment	Detritus, algae (16)
Diamesinae	P sediment (+ water?)	? (predatory + detritivorous spp.) (2)

[a]L = littoral, P = profundal.

[b]References: (1) Hanström 1972; (2) Merritt and Cummings 1978; (3) H. J. Johnson 1985; (4) Thompson 1978; (5) Pritchard 1964; (6) Folsom and Collins 1984; (7) Luecke and Litt 1987; (8) Vodopich and Cowell 1984; (9) Moore 1980; (10) Kajak and Dusoge 1970; (11) Wiederholm 1984; (12) Kajak and Dusoge 1967; (13) Titmus and Badcock 1981; (14) Walshe 1951; (15) Mattingly et al. 1981; (16) R. K. Johnson 1985.

or species level (e.g. Wiederholm 1983), with the exception of odonates, which, owing to their similarity in feeding habits, were only identified to the level of suborder or family (May 1933). Some samples were divided according to animal size or sex. To quantify surface adsorption of Hg, subsamples from some of the samples were rinsed in 0.5 M HCl, 0.1 M EDTA, or distilled water for 10 min before further treatment. Pooled samples of 1–442 individuals were weighed before and afer freeze-drying for 3 d. Samples of zooplankton, fish tissues, and sediment were also freeze-dried. Sediment carbon and nitrogen concentrations were determined with a Carlo-Erba CHN analyser.

Hg concentrations were determined by duplicate injection using an automated flow system for cold vapour atomic absorption spectrophotometry (Håkanson et al. 1990b), after acid digestion of 10–100 mg of dried sample in a mixture of concentrated H_2SO_4 + HNO_3 (2:1) for 1 h at 250°C under pressure in a teflon-sealed pyrex tube (Uhrberg 1982). Most digested samples were analyzed twice and the mean values calculated. The detection limit (3 SD) for a sample of 100 mg was 20 ng Hg·g dry weight $(dw)^{-1}$, and the coefficient of variation in regularly analyzed standard solutions was <2%.

The variability of Hg concentrations in macroinvertebrates was evaluated statistically by means of analysis of variance (ANOVA) and covariance (ANCOVA) to assess differences between lake types, regions, and taxa and to study the influence of water and sediment characteristics. In most analyses, Hg concentrations were transformed to log[Hg], partly to obtain normal distributions with similar variance from Hg concentrations which usually follow log-normal distributions within populations, and partly to apply linear statistics to multiplicative models which were considered most appropriate for comparing populations and their responses to environmental factors. Some calculations were based on all samples and others only on the main taxa that were encountered in most lakes and for which more than 10 observations with similar variability were available (Aeschnidae, Libellulidae, Zygoptera, *Asellus*, *Sialis*, *Procladius*, and *Chaoborus*). A few individual values (cf. numbers in parentheses in Table 3) were excluded from some statistical analyses if they referred to pooled samples from different periods (n = 3) or if they were considered unrepresentative because they either were at the detection limit (n = 6) or repeatedly gave abnormally large model residuals compared with most values (n = 4).

Results and Discussion

Taxonomic Composition

Profundal sampling provided almost exclusively dipteran larvae. In the littoral, larger animals such as odonates (Libellulidae, Aeschnidae, and Zygoptera), *Sialis lutaria* (Megaloptera) and *Asellus aquaticus* (Isopoda) were common. The taxonomic composition of samples from lakes of the same type but located in different regions was very similar, but differences were observed between lake types (Table 3). For instance, very few detritivorous dipteran larvae were found in the profundal zone of dystrophic lakes. The chironomids *Stictochironomus* and *Sergentia* were common only in the oligotrophic lakes, which agrees with Brundin's (1949) classification of *Stictochironomus rosenschoeldi* and *Sergentia coracina* as species characteristic of moderately deep oligohumic lakes. *Chironomus* spp. were most common in the mesotrophic lakes.

Hg Concentrations in Macroinvertebrates

Total Hg concentration in invertebrates ranged from <50 ng·g dw^{-1} in some *Sialis* and *Chaoborus* to

TABLE 3. Upper table: ranges of total Hg concentrations (ng·g dw^{-1}) in macroinvertebrates from eight Swedish forest lakes. Lower table: sampling occasions from which one or several samples of a taxon were analyzed, arranged in order of increasing Hg concentrations to show seasonal pattern (see Fig. 1 for numbering). In rare cases, animals from different periods were pooled, which is indicated by "+". Values which were considered uncertain or unrepresen- tative (see text) are shown in parentheses.

	Lake type and name							
	Acidic dystrophic		Neutral dystrophic		Mesotrophic		Oligotrophic	
Taxon	Bottentjärn	Skärhultsjön	Blacksåstjärn	Löjesjön	Loppesjön	Gårdsjön	Stensjön	Holmeshultasjön
Asellus aquaticus	336	202–394	63–257	199	150–224	71–169	163–250	270
Sialis lutaria	(<50) 59–75	120–238				50–133		34–47
Zygoptera	270–401	448	140–220	87–170	116–258	(<150)–116	77–150	49–130 (–2300)
Aeschnidae	273–593	192	254–329	219	140–209	221–345	97–225	395
Libellulidae	223–409	279–487	147–393	147–319	135–152	205–358	93–157	(<100)
Chaoborus	127–195	(<40) 65–79	56	42–124	91 (–347)	(<50)	(<50)	58
Procladius	163–173		131 (–332)			169	148	42–54
Demicryptochironomus			182					
Cryptochironomus				45				
Sergentia	365						707–4608	71–591
Stictochironomus							4604–5757	1003
Chironomus			297–722		130–1349	285–470		115–146
Diamesinae					65–118			
Asellus aquaticus	7	8 < 6 < 4 < 2	6 < 4 < 2 < 7 < 8 < 2 < 4	8	6 < 4 < 2	8 < 4	7 < 2	2 + 4 + 6 + 7
Sialis lutaria	(2<) 4 < 7	6 < 2				8 < 6		7 < 2 < 7
Zygoptera	8 < 6 < 4	4 + 6	2 < 8	2 < 8 < 7				
Aeschnidae	4 < 8 < 4 < 8 < 7 < 6	8	4 < 6	2	2 < 6	(4<) 8	2 < 8 < 6	6 < 8 < 4 < 7
Libellulidae	8 < 6 < 4 < 7 < 2 < 8 < 6 < 7	6 < 4	6 < 4 < 2 < 2	7 < 8 < 6	8 < 4 < 4 < 6	4 < 8 < 6	4 < 2 < 4 < 6 < 6 < 4 < 8 < 2 < 4 < 8	6
					2 < 4	4 < 8 < 6	6 < 2 < 7 < 8 < 4 < 6	7
Chaoborus	6 < 8 < 7	(4<) 2 < 8	2 + 7 + 8	4 < 8 < 2 < 7 < 6				
Procladius	7 < 6 = 8 < 4		6 (< 4)		6 (<2)	4	2	(4 + 6)
Demicryptochironomus			6 + 8			2 + 4 + 8	2	4 < 8 < 6
Cryptochironomus				4				
Sergentia	4						2 ≪ 4 ≪ 7	2 < 8 < 6
Stictochironomus							6 < 6	7
Chironomus			2 < 8 < 7		6 < 8 < 4 < 7 ≪ 2	6 < 2 < 8 < 4 < 2		8 < 8
Diamesinae					8 < 2 < 6 < 4 < 7			

>5000 ng·g dw^{-1} in some chironomid larvae (Table 3). The concentrations were thus highly variable and depended on the type of lake, the habitat, and the species. In general, the variations in Hg concentration were much larger in profundal animals than in littoral species. Animals living exclusively in the littoral zone, such as odonates and *A. aquaticus*, contained between 100 and 600 ng·g dw^{-1} (except for one extreme value of 2300 ng·g dw^{-1} in a sample of Zygoptera). *Sialis lutaria* typically had somewhat lower Hg content, 50–250 ng·g dw^{-1}. Similar low values were found for some profundal dipteran larvae (e.g. *Procladius* and *Chaoborus*) whereas very high concentrations (up to almost 6000 ng·g dw^{-1}) were measured in some other profundal chironomids (e.g. *Sergentia* and *Stictochironomus*).

Methyl mercury (MeHg) is the compound of greatest significance with respect to biomagnification of Hg in food webs. In the present study, the size of the macroinvertebrate samples was insufficient for analysis of MeHg. However, the proportion of MeHg in macroinvertebrates is probably similar to that in zooplankton from the same lakes, where MeHg typically accounted for 45–85% of total Hg, with a median of 65% (M. Meili, in prep.). Surma-Aho et al. (1986) determined that the proportion of organic Hg (derived from the operationally defined Magos method) in zoobenthos from natural lakes similar to our study lakes and from young reservoirs in Finland was 35–100% and about the same as in zooplankton. This is similar to the 23–100% (mean 76%) found in invertebrates from a remote mountain stream (Huckabee et al. 1979). Other studies also suggest that on average, more than half of the total Hg content in invertebrates consists of MeHg, although great variability has been reported.

Huckabee et al. (1979) also reviewed earlier results, partly from contaminated systems, and reported typical MeHg proportions in invertebrates of 50–80% of total Hg but also a variation from <1 to 100%. They proposed that surface adsorption and gut contents partly explained this variability. In this study, surface adsorption of Hg was examined by rinsing subsamples of *Chironomus* and Aeschnidae with either HCl, EDTA, or H_2O. No significant differences in Hg concentrations between animals from the different treatments were found, leading to the conclusion that surface-adsorbed Hg was not a major contributor to the total Hg content of the animals. To minimize the influence of intestinal content on the total body burden of Hg, at least 8 h (10–20°C) was allowed for the animals to void their guts. Widely varying gut clearance times have been reported for insect larvae. Ward and Williams (1986) considered 20 min adequate for chironomid larvae whereas Johnson et al. (1989) measured a gut passage time of 90 min in sediment-feeding *Chironomus plumosus* at 19°C. Bissonnette (1975) allowed 24–48 h for the animals to void their guts, and Verta et al. (1986) allowed 4–10 h. In natural systems without any direct discharge of Hg, such as the lakes in this study, concentrations in sediment are usually lower than in deposit feeders (cf. Tables 1 and 3), and the amount of Hg in the gut content will contribute little to the total body burden.

Relationship with Environmental and Ecological Factors

Lake character, climate, and atmospheric Hg deposition

Despite the wide range of Hg concentrations in macroinvertebrates, systematic differences among the lakes and the taxa were evident. To facilitate the comparison of Hg concentrations in zoobenthos from different lakes, typical values for each taxon in each lake were calculated as the product of overall contamination, a taxon-specific coefficient, and a lake-specific coefficient (Hg = k·TAXON·LAKE). These values were fitted from observed Hg concentrations by means of ANOVA, using log[Hg] as the independent variable (log[Hg] = log[k] + log[TAXON] + log[LAKE], model 6 in Table 4). The influence of both dependent variables was highly significant ($P < 0.0001$), and the model accounted for 62% of the total variance when including the main taxa ($n = 130$, $R^2 = 0.62$). When including all taxonomic groups in the calculation ($n = 164$), R^2 increased to 0.68, mainly as a result of the wider range of Hg concentrations among taxa.

From the fitted values for the main taxa (model 6), it is evident that Hg concentrations in lakes of the same type were similar (Fig. 2). This was tested by replacing the variable LAKE by the variables lake TYPE and REGION (model 7). In this analysis ($R^2 = 0.62$), TYPE was highly significant ($p < 0.0001$) whereas REGION proved to be an insignificant factor ($p = 1.0$), which confirmed the similarities among lakes of the same type. Both models revealed highest Hg concentrations in the acidic dystrophic lakes, followed by the neutral dystrophic lakes and the mesotrophic lakes which showed similar values. Lowest values were found in the oligotrophic lakes.

The similarity of the regions was surprising, since recent deposition of Hg is around 16 μg·m^{-2}·yr^{-1} in region S but only around 9 μg·m^{-2}·yr^{-1} in region C (Meili 1991c). Hg concentrations in sediments were also higher in region S (Table 1). In profundal sediments, a general correlation between Hg in zoobenthos and Hg in sediment could be expected, which would imply higher Hg concentrations in region S. However, highest concentrations in profundal animals were found in a lake from region C with intermediate Hg levels in the sediment (Stensjön). It could be argued that higher concentrations in zoobenthos in this region are caused by a higher degree of oxygen depletion in the bottom waters during winter as a result of the longer period of ice-cover. Anoxia appears to favour net methylation of Hg (Korthals and Winfrey 1987), thereby increasing its bioavailability. However, this would also imply that annual concentration maxima in profundal invertebrates would occur immediately after ice-out, which was only rarely the case (Table 3).

Lack of correlation between total Hg concentrations in sediment and in benthic animals has also been reported from other investigations (e.g. Kristensen 1982). Similarly, Jackson (1988) found no significant correlations between Hg in zoobenthos and total Hg, MeHg, or methylating activity in the sediment. Likewise, Bissonnette (1975) reported over a 50-fold variation in bioconcentration factors (Hg in chironomids/Hg in surface sediment, wet weight (ww)) across four lakes. It can be concluded that areal load or volumetric concentrations of Hg or MeHg are not the only factors regulating Hg concentrations in lacustrine invertebrates.

It appears most likely that regional differences in biodilution and trophic interactions counteract the effect of differences in atmospheric deposition of Hg between the regions (Meili 1991d). The importance of planktonic biodilution is supported by the sediment Hg/C ratios which were similar in both regions, in contrast with total Hg (Table 1). Trophic interactions may explain the findings that Hg concentrations in different invertebrate taxa were highly variable within the same habitat and that the concentrations in northern pike were higher or equal in region C compared with region S (Fig. 2). Differences among lakes in the relationship between Hg concentrations of northern pike and zoobenthos can possibly be ascribed to the mixed food sources of the prey fish of northern pike, comprising not only

TABLE 4. ANOVA and ANCOVA of log[Hg] in macroinvertebrates (ng·g dw^{-1}) with selected variables in eight Swedish forest lakes. In multivariate models, $p < 0.0001$ for all variables except where indicated, and p-values in parentheses refer to all taxa.

Model					Main taxa (n = 7)			All taxa (n = 13)		
No.	Var. 1	Var. 2	Var. 3	Var. 4	n	R^2	p	n	R^2	p
1	TAXON				130	0.34	0.0001	164	0.54	0.0001
2	LAKE				130	0.28	0.0001	164	0.08	0.07
3	TYPE				130	0.22	0.0001	164	0.02	0.32
4	REGION				130	0.19	0.03	164	0.03	0.04
5	PERIOD				124	0.01	0.89	158	0.02	0.46
6	TAXON	LAKE			130	0.62	0.0001	164	0.68	0.0001
7	TAXON	TYPE	REGION 0.97 (0.06)		130	0.62	0.0001	164	0.66	0.0001
8	TAXON	LAKE		PERIOD 0.31 (0.41)	124	0.65	0.0001	158	0.70	0.0001
9	TAXON	TYPE	REGION 0.62 (0.04)	PERIOD 0.37 (0.57)	124	0.64	0.0001	158	0.67	0.0001
10	TAXON	PH			130	0.43	0.0001	164	0.58	0.0001
11	TAXON	PH	REGION × PH 0.92 (0.13)		130	0.43	0.0001	164	0.59	0.0001
12	TAXON	logCOLOR (0.0002)			130	0.43	0.0001	164	0.58	0.0001
13	TAXON	logCOLOR (0.004)	REGION × logCOLOR 0.38 (0.42)		130	0.44	0.0001	164	0.58	0.0001

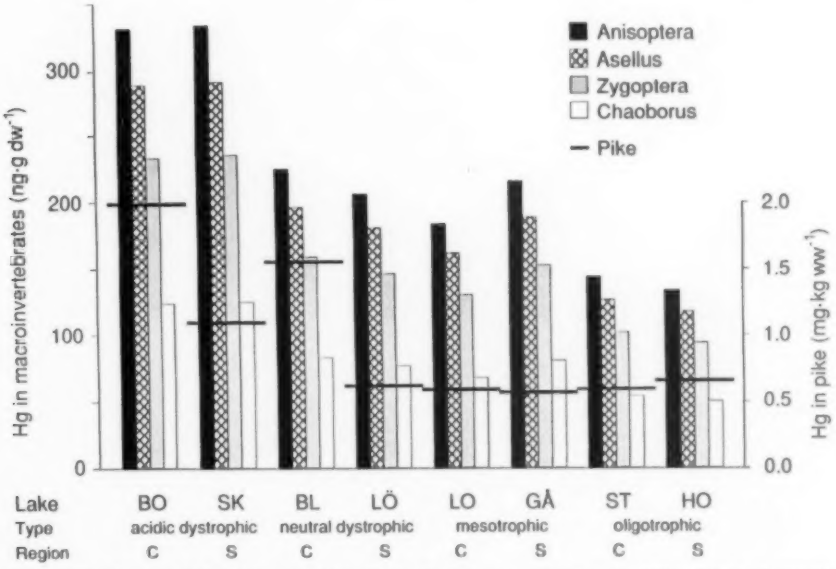

FIG. 2. Fitted values (ANOVA, model 6 in Table 4) of Hg concentrations in different taxa of macroinvertebrates found in all eight study lakes, and mean Hg concentration in the muscle tissue of 1-kg northern pike (*E. lucius*) in spring at a sex ratio of 1:1. See Table 1 for lake abbreviations.

zoobenthos but also zooplankton, fish fry, and plant matter (Meili 1991a). In addition, pelagic and benthic food chains may differ in Hg exposure and turnover (Meili 1991a; cf. Jackson 1991).

In boreal lakes, environmental conditions vary seasonally with respect to temperature, degree of thermal stratification, and supply of humic matter, nutrients, or acid from the watershed (Meili 1991a). The same is valid for the input of Hg (Iverfeldt and Johansson 1988; Mierle 1990; Meili et al. 1991) and MeHg (Lee and Hultberg 1990). In addition, seasonal fluctuations have also been observed in the concentration, formation, and degradation of MeHg in the water column and in sediments (Furutani and Rudd 1980; Korthals and Winfrey 1987; Parks et al. 1989). However, such fluctuations did not seem to affect Hg concentrations in zoobenthos in a consistent manner, since overall variations among periods were not statistically significant ($p > 0.3$, ANOVA models 8 and 9 in Table 4).

Water chemistry

Despite the lack of a general seasonal pattern of Hg concentrations in animals, the concentrations in individual invertebrate samples were correlated with pH and colour in water samples taken simultaneously at the same depth. In all taxa, Hg con-

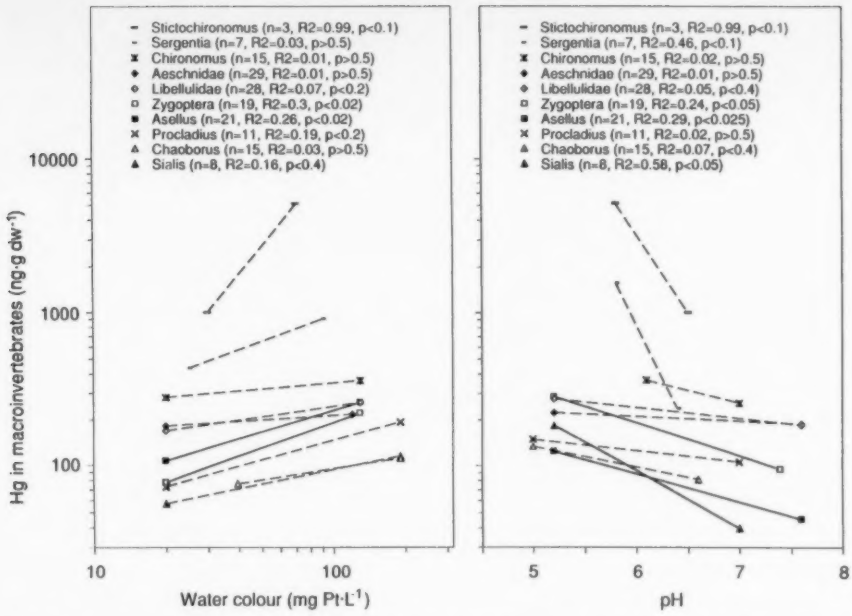

FIG. 3. Hg concentrations in different taxa of macroinvertebrates from eight Swedish forest lakes: relationship with pH and colour in the water column at sampling depth. The lines were derived from linear regression of log[Hg] on pH and log[colour], respectively. Nonsignificant regressions are indicated with broken lines.

centrations were high at low pH and at high colour (Fig. 3). Most regressions for single taxa were not statistically significant, but when pooling all or major taxa in an ANOVA (models 10 and 12 in Table 4), correlation of log[Hg] with pH and log(colour) became highly significant ($p < 0.0001$, $R^2 = 0.58$). The influence of these variables was not significantly different between the regions (ANCOVA, models 11 and 13 in Table 4), although region S was about twice as acidified as region C (SNV 1986).

As with the Hg concentrations in macroinvertebrates, mean Hg concentrations in the muscle of 1-kg northern pike were elevated in the acidic dystrophic lakes (Fig. 2; Table 1), which is in accordance with results from more comprehensive studies showing that Hg concentrations in northern pike and other fish usually correlate positively with lake dystrophy and acidity (Mannio et al. 1986; Verta et al. 1986; Håkanson et al. 1990a; Wren et al. 1991).

The positive correlations between Hg accumulation in biota and the dystrophy or acidity of lake water may be mediated by effects on both the chemical speciation of Hg and the physiology of the organisms. It is well established that both the interaction of Hg with organic matter and the transformation of mercurials are affected in different ways by the ambient pH (e.g. Thanabalasingam and Pickering 1985; Winfrey and Rudd 1990). Methylation and demethylation in the water column, at the sediment surface, and in subsurface sediments may increase or decrease as a result of changes in pH and concentration of dissolved organic matter (Winfrey and Rudd 1990; Miskimmin et al. 1992). From a field study in remote seepage lakes, where a negative correlation between Hg content of yearling yellow perch (*Perca flavescens*) and lake water pH was found (Bloom et al. 1991), it was concluded that the dominant effect of pH was on the production of MeHg (inversely related to pH) rather than on its bioaccumulation. However, laboratory experiments showed that the uptake of not only $HgCl_2$ but also MeHgCl by mayfly nymphs (*Hexagenia rigida*) increased with decreasing pH (Saouter et al. 1989) and that the diffusion fluxes of these Hg species over planar lipid bilayers were higher at pH 5.0 than at pH 8.5 (Boudou et al. 1991).

Taxa

Within lakes, wide differences in Hg concentrations were found between taxa (Table 3). General relationships among taxa were evaluated with the ANOVA described above (model 6 in Table 4; Fig. 2). To assess the relationship among taxonomic groups across lakes using data from all lakes, Hg concentrations were normalized for differences among lakes. For this purpose, a reference concentration was calculated for each lake from the fitted model values for Anisoptera (mean of Hg concentations in Aeschnidae and Libellulidae, which were not significantly different (ANOVA, $p = 0.59$, $n = 57$)). All observed Hg concentrations were then normalized by calculating their ratio to the reference value from the respective lake. The geometric means of normalized concentrations were also calculated for each taxon. Anisoptera were chosen as a reference because they are common in most lakes, easily distinguished from other animals, and can be collected throughout the ice-free season from the shore without sophisticated equipment. In addition, little effort is required to provide enough material for Hg analysis, Hg concentrations are comparatively high, and seasonal and size-dependent variations are negligible (see below). Moreover, species of this suborder have a similar feeding behaviour and life cycle.

The resulting mean Hg concentrations for the examined taxa differed 100-fold (Fig. 4). This wide range can partly be explained by ecological differences (see below). The geometric range (max/min) within a taxon was fairly constant, typically

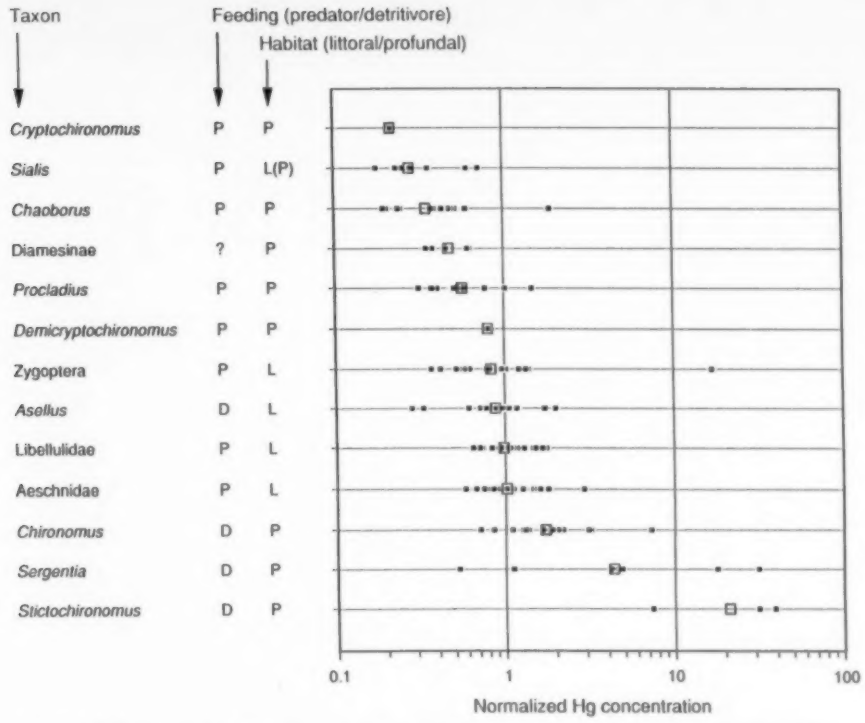

FIG. 4. Hg concentrations in different taxa of macroinvertebrates from eight Swedish forest lakes, shown on a log scale as normalized values relative to fitted values (ANOVA) for larvae of Anisoptera (Odonata) from the respective lake (cf. Fig. 2). Open squares show fitted values or geometric means of normalized concentrations.

around 3–4 (>90% of the values for the major taxa were within 50 and 200% of the geometric mean).

The concentration ranges within lakes depended on the taxonomic composition of the macroinvertebrate communities. While the ratios of mean Hg concentrations between given taxa were similar in different lakes, Hg concentration ranges were lake type specific and depended on the occurrence of animals with high Hg concentrations. Among the taxa which were found in most lakes (main taxa), the concentrations were highest in the Libellulidae and Aeschnidae from the acidic dystrophic lakes, up to 600 ng·g dw^{-1} (Table 3; Fig. 5). However, when considering all taxa, maximum Hg concentrations were almost 6000 ng·g dw^{-1}. Highest values were found in the profundal chironomids *Stictochironomus* and *Sergentia*, which were common only in the two oligotrophic lakes where overall concentrations were lowest (Table 3; Fig. 2–5). The third profundal group with occasionally high Hg levels (up to 1400 ng·g dw^{-1}), *Chironomus*, was most common in the mesotrophic lakes. Low concentrations (<150 ng·g dw^{-1}) were mostly found in *Sialis* and *Chaoborus*. These findings show that the analysis of aggregated invertebrate samples may induce significant bias in comparative studies of lake contamination.

Life cycle

Considerable seasonal variations in Hg concentrations were found in some groups of animals but not in others. Hg in *A. aquaticus* showed similar seasonal variations in the different lakes, with higher values in spring and lower values in late summer. The low concentrations coincided with the annual appearance of a new generation in July, when the average individual weight was lowest (Fig. 6), and the normalized Hg concentrations were thus weakly but positively correlated with the average weight of the individuals ($p \approx 0.1$, $n = 21$), (Fig. 7). From two samples collected in Blacksåstjärn, egg-carrying females were isolated and it was found that the Hg concentrations were higher than in the rest of the sample which consisted mainly of males. As females were heavier at this stage, this agrees with the positive relationship between Hg concentration and body size.

Concentrations in profundal dipteran larvae fluctuated to a variable extent. In genera with low concentrations (*Chaoborus*, *Procladius*, and Diamesinae), the variations were small in absolute terms whereas the geometric variation (on a log scale) was similar to that in most other taxa (Fig. 4 and 5). In some lakes, Hg in *Chaoborus* decreased to a minimum before the animals emerged (June–July). Hg in the chironomid larvae with high concentrations varied considerably, e.g. the geometric range (max/min) was >6 for *Sergentia* in both lakes where it was found more than once, but no correlation with body weight was found (Fig. 7). The wider range is likely to reflect spatial and temporal changes in the environment affecting not only the speciation and availability of Hg, but also the behaviour and metabolism of animals, e.g. as a reaction to hypoxia.

The Hg concentration in Aeschnidae and Libellulidae varied little and not systematically with time (Fig. 5), and no correlation was found with the average weight of individuals despite a range of 3–280 mg dw (Fig. 7). Some variations in Hg concentrations of Zygoptera were found, but the patterns among

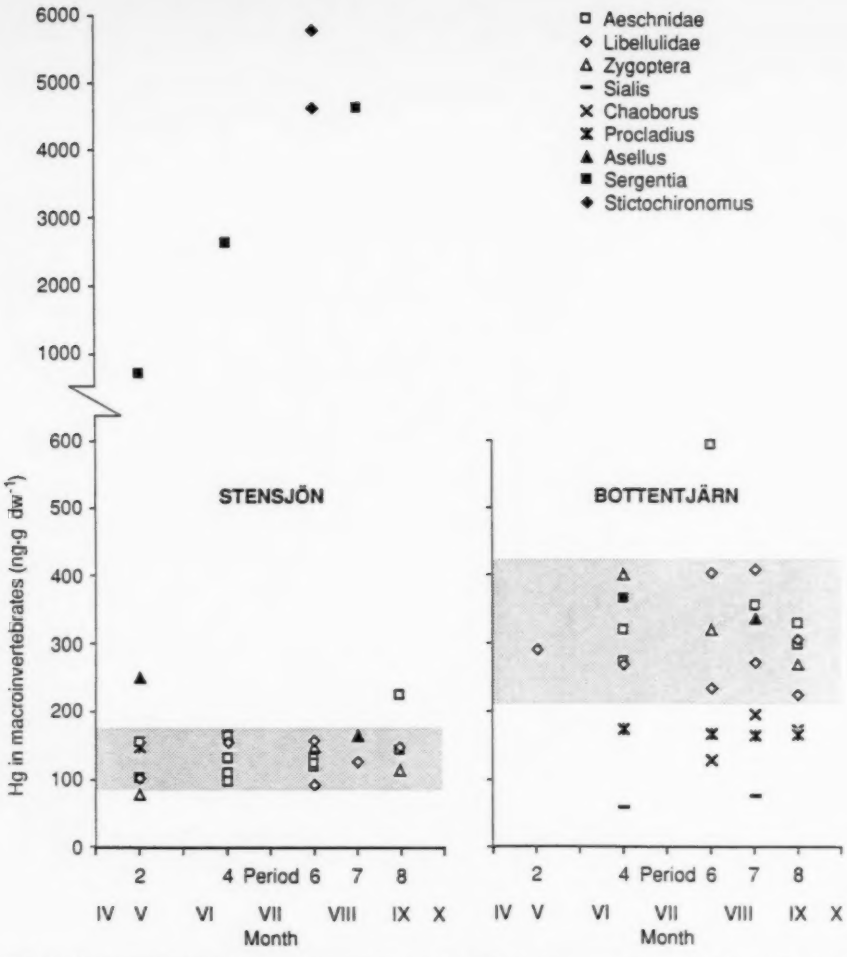

FIG. 5. Hg concentrations in macroinvertebrates during different seasons (cf. Fig. 1) in acidic dystrophic Lake Bottentjärn and in oligotrophic Lake Stensjön (central Sweden). Shaded areas highlight the typical range for Anisoptera (Aeschnidae and Libellulidae).

lakes were not consistent nor did they correlate with changes in the weight of individuals.

When considering all predatory taxa, Hg concentrations were correlated with mean individual weight across taxa, but not within taxa (Fig. 7). Apparently, Hg concentrations do not increase during ontogeny, which suggests that the turnover of Hg in organisms is a dynamic process which is little affected by exposure time. As a rule, larger predators feed on larger prey organisms and are thus more likely to belong to a higher trophic level and to accumulate more Hg because of the biomagnification of MeHg in food chains, similar to the patterns found in fish communities (Meili 1991a, 1991b). In contrast, for many other metals for which surface adsorption is especially important, there is an inverse relationship between metal concentration and body size (Smock 1983).

Habitat and feeding behaviour

In profundal animals, Hg concentrations reached values over 10 times higher than in any of the littoral taxa (Fig. 4). Among littoral animals, Hg concentrations were highest in the large predatory odonates (Aeschnidae and Libellulidae). Among the smaller littoral animals, the concentrations were high in the detritivore *Asellus*, somewhat lower in predatory Zygoptera, and much lower in predatory *Sialis* (Fig. 2, 4, and 6). These comparisons provide a first indication for the importance of considering feeding habits (Table 2) when comparing animals within a habitat.

Even stronger evidence for the impact of feeding habit on the Hg accumulation is provided when only profundal dipteran larvae are considered. In these animals, the lowest Hg concentrations were found in predators (typically <40–200 ng·g dw^{-1}) (Table 3; Fig. 4), among which *Procladius* and *Chaoborus* were most frequently found. *Demicryptochironomus* and *Cryptochironomus*, which were found only once and contained concentrations of only 182 and 45 ng·g dw^{-1}, respectively, are also predators (Table 2). The highest Hg concentrations among all taxa were found in detritivorous chironomid larvae (*Stictochironomus*, *Chironomus*, and *Sergentia*). In contrast, another genus of red chironomids from the Diamesinae family, which was common in Loppesjön and was collected at the same time and place as *Chironomus*, contained

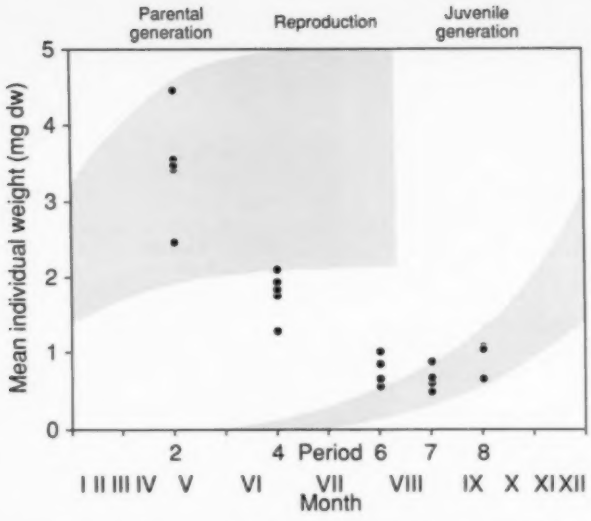

FIG. 6. Seasonal changes in the average individual dry weight of *A. aquaticus* in pooled samples (≥15 individuals) from eight Swedish forest lakes. Shaded areas highlight the shift of generations in July (cf. Andersson 1969).

very low levels of Hg (Table 3; Fig. 4). The foraging behaviour of these animals is unknown, but since they had low concentations of Hg similar to other predatory dipterans, it may be speculated that they belonged to one of the predatory genera in the family.

The Hg concentrations of *Chironomus* in the two mesotrophic lakes covaried seasonally, if one extreme value in spring is disregarded. Animals analyzed for Hg were only determined to genus, but the samples taken for quantitative taxonomic analysis showed that this genus was represented by two deposit feeders, *C. anthracinus* and *C. plumosus*. In both lakes, *C. plumosus* dominated the biomass in the samples from periods 2, 4, and 8 whereas *C. anthracinus* dominated in period 6. A covariation was found between the dominance of *C. anthracinus* and low Hg concentration in *Chironomus* spp. R. K. Johnson (1985) measured C/N ratios in the anterior gut contents of the same two species of *Chironomus* as found in this study, and in the ambient sediment. He found highest C/N ratios in the sediment, somewhat lower ratios in the anterior gut of *C. anthracinus*, and the lowest ratio in the anterior gut of *C. plumosus*. This indicates different foraging strategies among detritivores within the same habitat, with *C. plumosus* ingesting fairly fresh organic material probably at the sediment surface, while *C. anthracinus* appears to be a less selective deposit feeder. The apparent difference in Hg concentration in these two species indicates that recently sedimented organic material may contain more Hg, or at least more bioavailable Hg (cf. Jackson 1986), maybe also more Hg-methylating bacteria (Korthals and Winfrey 1987). At the sediment–water interface with its steep gradients in oxygen concentration, food quality, bacterial activity, and methylation potential, minor differences in feeding strategy may result in large differences in Hg uptake.

The large variations in Hg concentrations among profundal dipterans as well as among littoral predators strongly suggest that feeding behaviour is of primary importance in controlling the Hg concentations in the benthic fauna. Our results agree with those from earlier investigations. Göthberg (1983) measured similar Hg concentrations (although reported as wet weight) in animals from a lake situated close to region S. *Sialis* and *Procladius* contained less Hg than *Asellus*, while Chironomini contained more. Similar relationships between taxa were also found by Särkkä (1979) in a contaminated Finnish lake, and pooling of species into functional groups resulted in the

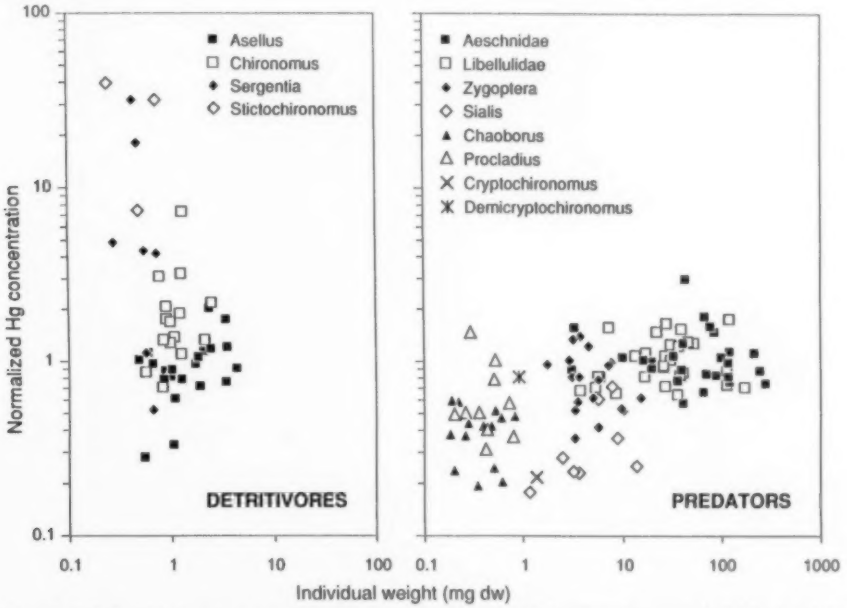

FIG. 7. Relationship between normalized Hg concentrations (cf. Fig. 4) and average individual weight in samples of macroinvertebrates from eight Swedish forest lakes.

following general patterns: profundal zoobenthos contained more Hg than littoral zoobenthos, and herbivorous and detritivorous insects contained more than predatory insects. Jernelöv and Lann (1971) and Armstrong and Hamilton (1973) found much higher concentrations, but comparable relationships, in heavily contaminated systems. In the latter study, Hg in sediment feeders ranged between 200 and 20 000 ng·g wet weight^{-1}, Hg in animals feeding on suspended material between 100 and 1000 ng·g wet weight^{-1}, and Hg in predators between 100 and 440 ng·g wet weight^{-1}.

Sediment as a Substrate

Sediment is the substrate of many collectors, and food quality, measured as growth rates of such animals, is related to the organic content of the sediment (Mattingly et al. 1981). A low nutritive quality necessitates a higher ingestion rate to achieve growth and results in a greater exposure to Hg for a given concentration in the food. The total Hg content of the sediment (preferably related to dry weight) may to some extent reflect the degree of contamination in a lake, but the exposure of nonselective collectors and filter feeders is probably better reflected by the Hg content of the organic fraction of the sediment. Accordingly, no correlation was found between Hg accumulation in *Chironomus riparius* grown in different types of lake sediment and the total Hg concentration in the ambient sediment whereas a positive correlation was found between the proportion of NaOH-extractable ("humus-bound") Hg in the sediments and the bioconcentration of Hg by the chironomids (Parkman et al. 1991). Also, Langston (1982) found positive correlations between the Hg/organic matter ratio in sediments and the Hg content in two marine bivalves. In the present investigation, a positive trend was found in the correlations between Hg/C and Hg/N in sediment and Hg in Anisoptera larvae. As these animals are predators and live on macrophytes rather than on the sediment surface, this trend is probably a result of either trophic interactions or causal relationships between sediment chemistry, water chemistry, and Hg exposure of animals in general (cf. Meili 1991d) rather than of direct interactions between sediment and Anisoptera. In contrast, the detritivorous chironomids, which are more directly dependent on sediment conditions, showed a weak negative correlation with Hg/C and no correlation with Hg/N in sediments.

The bioavailability of sediment-bound Hg is affected by abiotic factors such as pH (see above) and temperature. Jackson (1988) found a negative correlation between Hg in chironomids and sampling depth and a positive correlation with the temperature of bottom water. He concluded, consistent with our observations, that the depth-related factors which controlled the uptake of Hg by animals was the nature of the ingested sediment and not the temperature. For instance, the Hg content of chironomids decreased with increasing concentrations of Fe (extractable with citrate/dithionite) in the sediments and with an increasing amount of C in the organic fraction of the sediment. Unfortunately, these samples were taken from different lakes and the animals identified only as chironomids. The variations could therefore have been a result of differences in the species composition of the samples.

The occasionally very high concentrations of Hg in *Sergentia* and *Stictochironomus* may partly be a result of high methylation rates in their surroundings. *Sergentia* build long tubes and have been observed feeding from the bottom of these tubes (Walshe 1951). In the oligotrophic lakes, where these genera were encountered, the oxycline is most probably below the sediment surface, and burrowing animals might reach the interface between oxic and anoxic sediment, where methylation of Hg is probably most active (Korthals and Winfrey 1987).

Conclusions

Hg concentrations in lacustrine macroinvertebrates are highly variable, and this can be ascribed to differences in lake type, ecological niches, and species-specific seasonalities. In profundal detritivores, Hg concentrations can reach very high levels, sometimes higher than in fish. Food quality appears to be the most important factor controlling partitioning of Hg in the different taxa of the invertebrate community within a lake. However, high levels in detritivores from the profundal but not from the littoral, as well as the differences between lakes, may be caused by abiotic and microbial processes such as speciation of inorganic Hg and formation of MeHg.

Since concentrations of Hg are considered to increase along food chains (biomagnification), it was surprising to find higher Hg concentrations in detritivorous genera such as *Sergentia* and *Asellus* than in predatory genera such as *Procladius* and *Sialis*. A possible explanation would be a high proportion of inorganic Hg in detritivorous animals; little of this Hg would be taken up by predators, since MeHg is bioaccumulated much more efficiently than inorganic Hg in most animals (e.g. Riisgård and Famne 1986; Saouter et al. 1989). However, currently available data on the proportion of MeHg in different taxa of macroinvertebrates are too limited to provide unequivocal support for this hypothesis.

It is difficult to determine to what extent empirical relationships are a result of direct trophic interactions and to what extent they are established by environmental conditions which are shared by all organisms in a lake. Whereas the Hg concentration of predatory animals is partly governed by the trophic structure and dynamics of the food web, Hg concentrations in lacustrine animals at low trophic levels are probably more directly dependent on abiotic and microbial processes (Jackson 1988). Because of their rapid metabolic and ecological turnover, these organisms are likely to respond more rapidly to changes in environmental conditions which affect the supply or bioavailability of Hg in lakes. It is evidently important to study both the ecology of benthic animals and the ambient chemical conditions simultaneously to explain differences and changes in Hg content. Future research should be focused on the speciation and bioavailability of Hg in sediments and the bioaccumulation of Hg in benthic animals with different feeding ecology and sediment environments. An equally important and neglected aspect is the turnover of Hg within invertebrate food webs. In a wider perspective, studies of benthic processes and partitioning of Hg in benthic food was are necessary to obtain a better understanding of Hg accumulation in fish.

Acknowledgements

We thank everyone who assisted in sampling, especially Björn Söderbäck. Mikael Östlund conducted all mercury analyses using the equipment of Roland Uhrberg (MeAna), and Lars Eriksson and Richard Johnson (SNV) provided taxonomic expertise and ecological advice.

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Prerelease Stratification in Tag-Recovery Models with Time Dependence

Robert M. Dorazio

U.S. Fish and Wildlife Service, National Fisheries Research Center, Box 700, Kearneysville, WV 25430, USA

Dorazio, R. M. 1993. Prerelease stratification in tag-recovery models with time dependence. Can. J. Fish. Aquat. Sci. 50: 535–541.

A statistical model is developed for estimating time-dependent survival and recovery rates of tagged populations that are stratified into groups at release. For populations of fish, the groups might include different sexes or stocks, or they might be distinguished by the location or method of capture. In the model developed here, survival and recovery rates are formulated as linear combinations of parameters that include the effects of time, group, and time–group interactions. Methods of testing the statistical significance of these potential sources of variation are described and illustrated with the recoveries of different stocks of anadromous striped bass (*Morone saxatilis*). For the striped bass data, elimination of time- and stock-specific sources of variation substantially improved the precision of survival estimates without introducing important bias. During 1988–89 the average survival of both stocks was 0.78 (SE = 0.04). Calculations of statistical power indicate that stock-specific differences in survival of ±0.2 could have been detected about 50% of the time, given the low rates of recovery and numbers of fish released.

On met au point un modèle statistique qui sert à l'estimation de la survie et du taux de récupération, en fonction du temps, de populations baguées qui sont stratifiées en groupes au moment de leur mise en liberté. Dans le cas de populations de poissons, les groupes pourraient être constitués selon le sexe ou le stock, ou encore la distinction pourrait porter sur l'emplacement géographique ou la méthode de capture. Dans le modèle élaboré ici, les taux de survie et de récupération sont formulés en termes de combinaisons linéaires de paramètres qui comprennent notamment les effets de durée, de groupe et de combinaisons d'effets de temps et de groupe. Les méthodes de vérification de la signification statistique de ces sources potentielles de variation sont décrites et illustrées au moyen de taux de récupération de différents stocks de Bar d'Amérique (*Morone saxatilis*) constituant différents stocks. Quant aux données sur le Bar d'Amérique, on observe que l'élimination des sources de variation spécifiquement liées au temps et aux stocks permet d'améliorer considérablement la prévision des estimations de la survie sans qu'il y ait introduction d'une erreur systématique importante. Au cours de 1988–1989, la survie moyenne des stocks était de 0,78 (erreur type de 0,04). Les calculs de puissance statistique indiquent que sur le plan de la survie, des différences spécifiques à des stocks de plus ou moins 0,2 auraient pu être détectées environ une fois sur deux compte tenu du faible taux de récupération et du nombre de poissons remis en liberté.

Received February 28, 1992
Accepted September 3, 1992
(JB421)

Reçu le 28 février 1992
Accepté le 3 septembre 1992

Large-scale, tag-recovery programs with fish populations often include releases of several classes or groups of fish over multiple years. Common examples of such prerelease strata include fish of different species, stocks, sexes, or sizes. The tagged population also might comprise the "controls" and "treatments" of experiments designed to estimate the effects of various treatments on survival or recovery rates. Such tagging experiments are especially common with hatchery-reared fish, which can be raised or stocked under different experimental regimes and released in large numbers (e.g. Johnson et al. 1990).

Prerelease stratifications of tag-recovery data can have important implications for the estimation of survival and recovery rates. Nichols et al. (1982) and Pollock and Raveling (1982) have shown that if rates actually vary among strata and strata are ignored by pooling recoveries, the estimated average rates of survival and recovery can be severely biased. The direction and magnitude of the biases depend on the level of among-stratum heterogeneity in survival and recovery and on the proportion of fish tagged and released in each stratum.

In a general review of statistical methods for estimating survival and recovery rates of tagged populations, Brownie et al. (1985) recommended that the recoveries of each stratum be analyzed separately when heterogeneity is detected. However, fish of different strata may experience some rates of similar magnitude and others that vary by stratum. For example, the survival of males and females might be similar, but sex-specific differences in migratory behavior could lead males and females to be recovered in locations that differ in tag reporting and apparent recovery rates. In such situations, separate analyses of each stratum's tag recoveries would include estimation of unneeded parameters and loss of precision.

Analyses of tag-recovery data stratified into groups at release therefore could be improved by using a statistical model that is sufficiently general to include the most likely sources of variation in its parameters but can be constrained to examine the effects of time, group, or time–group interactions on estimates of survival and recovery. Considerable progress has been made along these lines to advance the original capture–recapture methodology of Cormack (1964), Jolly (1965), and Seber (1965). This is due, in part, to the availability of software such as SURVIV (White 1983). In a thorough review of the advances in statistical methodology, Lebreton et al. (1992) provided case studies that illustrate the ways that prerelease strata (therein

TABLE 1. Representation of data for a 5-yr study involving two groups of fish tagged and released during the first 3 yr of the study ($m = 2$, $k = 3$, and $l = 5$).

Year released	Number released	Year of recovery 1	2	3	4	5
		Group 1 recoveries				
1	N_{11}	R_{111}	R_{121}	R_{131}	R_{141}	R_{151}
2	N_{21}		R_{221}	R_{231}	R_{241}	R_{251}
3	N_{31}			R_{331}	R_{341}	R_{351}
		Group 2 recoveries				
1	N_{12}	R_{112}	R_{122}	R_{132}	R_{142}	R_{152}
2	N_{22}		R_{222}	R_{232}	R_{242}	R_{252}
3	N_{32}			R_{332}	R_{342}	R_{352}

referred to as "multiple data sets"), time dependence, age dependence, and time covariates have been included in capture–recapture models. Owing to the wide range of available models, analysts of capture–recapture data can probably find a biologically realistic model that adequately fits their data. Emphasis therefore has shifted to testing hypotheses about the effects of various factors, such as time or age dependence, on survival or recapture rates. The goals of such testing are to minimize bias and maximize precision of parameter estimates by starting with a model that includes the major sources of variation in its parameters and systematically eliminating all the statistically insignificant sources of variation. Burnham and Anderson (1992) have referred to this as searching for a set of parsimonious models that fit the data and provide limited inferences about variation in survival and recapture rates.

Here, I develop a model for estimating time-dependent survival and recovery rates of tagged populations that are stratified into groups at release. Survival and recovery rates are formulated as linear combinations of parameters that include the effects of time, group, and time–group interactions. I show that this formulation is equivalent to the time-dependent and age-independent estimators described by Brownie et al. (1985) for the analysis of unstratified populations. The advantages of the newly formulated estimators are described and illustrated with recoveries of different stocks of striped bass (*Morone saxatilis*) that were tagged and released in geographically distinct areas along the east coast of the United States.

Statistical Model

Following the notation of Brownie et al. (1985), consider the situation in which m distinct groups of fish are captured, tagged, and released at roughly the same time for k successive years. The interval between releases need not be annual, but this is common in studies with fish or migratory birds. Recoveries of tagged fish occur during the years between releases and may also continue afterwards; therefore, let l be the total number of years in which tagged fish are recovered. The annual numbers of releases and recoveries of tagged fish are conveniently arranged in matrices (Table 1) of N_{ig}, the number of fish of group g released at the time of tagging in year i ($i = 1, \ldots, k$; $g = 1, \ldots, m$), and R_{ijg}, the number of fish of group g released at the time of tagging in year i and recovered during year j ($i = 1, \ldots, k$; $j = i, \ldots, l$; $g = 1, \ldots, m$).

The fully parameterized tag-recovery model developed here includes both time and group dependence of survival and recovery rates. Let f_{jg} be the probability that fish of group g are recovered and reported during year j ($j = 1, \ldots, l$; $g = 1, \ldots, m$) and S_{jg} be the probability that fish of group g survive from the time of tagging in year j to the time of tagging in year $j + 1$ ($j = 1, \ldots, l - 1$; $g = 1, \ldots, m$). This parameterization implies that survival and recovery are independent of the year in which fish are released. Recovery parameters are assumed to vary only with the year in which fish are recovered and the group to which they belong. Survival parameters are assumed to vary only with the year in which fish are alive and the group to which they belong.

To assist testing of hypotheses about the effects of time or group on survival or recovery rates, the probabilities f_{jg} and S_{jg} are formulated as linear combinations of parameters that include the effects of time, group, and time–group interactions. Specifically, recovery probabilities are expressed as follows:

$$f_{j1} = f_{11} + \alpha_j$$
$$f_{1g} = f_{11} + \beta_g$$
$$f_{jg} = f_{11} + \alpha_j + \beta_g + \delta_{jg}.$$

In these equations, f_{11} is the probability that fish of group 1 are recovered and reported during year 1, α_j is the effect of year j on f_{11} ($j = 2, \ldots, l$), β_g is the effect of group g on f_{11} ($g = 2, \ldots, m$), and δ_{jg} is the effect of a year j – group g interaction on f_{11}. The logical range of f_{11} extends from 0 to 1 because it is a probability. The "effects" parameters, however, may be positive or negative depending on the magnitude of the differences between f_{11} and f_{jg}. Survival probabilities are expressed similarly:

$$S_{j1} = S_{11} + \mu_j$$
$$S_{1g} = S_{11} + \tau_g$$
$$S_{jg} = S_{11} + \mu_j + \tau_g + \sigma_{jg}.$$

In these equations, S_{11} is the probability that fish of group 1 survive from the time of tagging in year 1 to the time of tagging in year 2, μ_j is the effect of year j on S_{11} ($j = 2, \ldots, l - 1$), τ_g is the effect of group g on S_{11} ($g = 2, \ldots, m$), and σ_{jg} is the effect of a year j – group g interaction on S_{11}.

To estimate the parameters of this model, I make the usual assumptions of tag-recovery models (Pollock and Raveling 1982), including independence of fates of individual fish; therefore, the recoveries of any cohort of fish tagged in year i and belonging to group g are modeled as multinomial random variables:

$$P(\{R_{ijg}: j = 1, \ldots, l+1\}) = \binom{N_{ig}}{\{R_{ijg}\}} \prod_{j=i}^{l+1} \pi_{ijg}^{R_{ijg}}$$

in which π_{ijg} is the probability that fish of group g and tagged in year i are recovered in year j ($i = 1, \ldots, k$; $j = i, \ldots, l$; $g = 1, \ldots, m$) and $\pi_{i,l+1,g}$ and $R_{i,l+1,g}$ are the probability and number, respectively, of fish of group g that are released in year i and never recovered, i.e.

$$\pi_{i,l+1,g} = 1 - \sum_{j=i}^{l} \pi_{ijg}$$

and

$$R_{i,l+1,g} = N_{ig} - \sum_{j=i}^{l} R_{ijg}.$$

In the fully parameterized model with time- and group-specific survival and recovery rates, π_{ijg} is defined as follows:

$$\pi_{ijg} = \begin{cases} f_{jg}, & j = i \\ f_{jg} \prod_{h=i}^{j-1} S_{hg}, & i < j \leq l. \end{cases}$$

Model parameters are estimated by maximizing the likelihood function L of the data, which equals the product of the independent multinomial likelihoods defined earlier:

$$L = \prod_{g=1}^{m} \prod_{i=1}^{k} P(\{R_{ijg}: j = 1, \ldots, l+1\}).$$

Maximization of L is easily accomplished with the FORTRAN program SURVIV (White 1983). In addition to the data matrices (Table 1), SURVIV requires FORTRAN expressions for the probabilities π_{ijg} formulated in terms of model parameters. SURVIV then numerically determines the parameter estimates that maximize L and provides an estimate of the asymptotic covariance matrix of the estimates. I developed a computer program (RECOVERY) that provides appropriately formatted input to SURVIV, including the probabilities π_{ijg} expressed in terms of f_{11}, α_j, β_g, δ_{jg}, S_{11}, μ_j, τ_g, and σ_{jg}. RECOVERY also contains a general interface for input or output of stratified tag-recovery data and is available on request.

Hypothesis Testing and Model Selection

Survival and recovery rates are formulated as linear combinations of time, group, and time–group effects to facilitate tests of the statistical significance of these potential sources of variation. Tests are conducted in a manner that resembles stepwise testing of hypotheses in log-linear, statistical models (Bishop et al. 1975; Lebreton et al. 1992, p. 84). Beginning with the fully parameterized model, the σ_{jg} parameters are eliminated to form a reduced model that excludes the effects of time–group interactions on survival rates. This reduced model is then compared with the fully parameterized model using a likelihood ratio test. If the reduced model is not rejected, this model is further constrained by eliminating each of the main effects parameters μ_j and τ_g separately. Likelihood ratio tests are used again to determine if either of the main effects parameters can be eliminated. If only one of the main effects parameters can be eliminated, the significance of the remaining main effect can be tested further in the final step of the backward elimination of survival rate parameters. In this step, a likelihood ratio test is used to compare a model with the parameters of the remaining main effect against a model of constant survival. Once a satisfactory model has been identified for survival rates, the backward elimination scheme is repeated for recovery rate parameters to determine if the effects of time, group, or time–group interactions are significant sources of variation in recovery rates.

TABLE 2. Summary of parameter constraints associated with various models for survival and recovery rates.

Model notation	Model description	Parameter constraints
$f_{T\cdot G}$	Time- and group-dependent recovery rates	None
f_{T+G}	Between-group parallelism in time-dependent recovery rates	$\delta_{jg} = 0$
f_G	Group-dependent recovery rates	$\delta_{jg} = 0$, $\alpha_j = 0$
f_T	Time-dependent recovery rates	$\delta_{jg} = 0$, $\beta_g = 0$
f	Constant recovery rate	$\delta_{jg} = 0$, $\alpha_j = 0$, $\beta_g = 0$
$S_{T\cdot G}$	Time- and group-dependent survival rates	None
S_{T+G}	Between-group parallelism in time-dependent survival rates	$\sigma_{jg} = 0$
S_G	Group-dependent survival rates	$\sigma_{jg} = 0$, $\mu_j = 0$
S_T	Time-dependent survival rates	$\sigma_{jg} = 0$, $\tau_g = 0$
S	Constant survival rate	$\sigma_{jg} = 0$, $\mu_j = 0$, $\tau_g = 0$

During model selection the elimination of survival rate parameters should precede the elimination of recovery rate parameters when most of the recovered fish are harvested or otherwise removed from the population. Although this concentrates most of the power on tests of recovery rates, it reduces the chances of accepting an unrealistic model in which survival rates vary among times or groups while harvest and recovery rates remain constant. This could occur, of course, but only if the differences in harvest rates associated with differences in survival are offset by opposing differences in tag reporting rates. This coincidence of events seems unlikely. Models with variable rates of survival and constant rates of recovery still might be appropriate, however, in some situations. Suppose fishing regulations change with time to permit catch-and-release, but not harvest, in one time interval that is followed by a second interval in which both methods of fishing are permitted. In addition, assume that all the tagged fish that are caught, reported as recovered, and released alive cannot be recaptured because their tags are removed during the first capture, a common practice in studies with fish populations. Under these circumstances, recovery rates conceivably could remain constant with time while survival rates decrease because more of the recoveries during the second time interval represent fish that are removed from the population as harvest. Of course, recovery rates also might increase if the relaxation of harvest restrictions attracts greater fishing effort. Therefore, for situations in which it seems reasonable to consider models with variable rates of survival and constant rates of recovery, model selection should begin with the backward elimination of recovery rate parameters. This concentrates most of the power on tests of survival rates, which are usually of greater biological importance anyway (Lebreton et al. 1992, p. 84).

The various models formed by eliminating one or more of the effects parameters can be summarized in a compact notation (Table 2) recommended by Lebreton et al. (1992, p. 118). For example, $S_{T\cdot G}$ indicates a model for survival that includes the effects of time, group, and time–group interactions whereas S_{T+G} indicates a model in which survival rates vary with time and group but the temporal pattern of variation is similar among groups. Such "parallelism" in survival rates implies that the

model S_{T+G} contains no time–group interactions (i.e. $\sigma_{jg} = 0$). Using this notation, $(f_{T\cdot G}, S_{T\cdot G})$ specifies a model that is mathematically equivalent to model 1 of Brownie et al. (1985) for estimating time-dependent survival and recovery rates separately for each group. Estimates obtained by using models 2 and 3 of Brownie et al. for each group are likewise equivalent to those obtained from models $(f_{T\cdot G}, S_G)$ and (f_G, S_G), respectively. The formulation of time and group dependence developed here is a generalization of the age-independent models developed by Brownie et al. Use of linear combinations of parameters does not alter the magnitudes of the temporal or group effects estimated by the methods of Brownie et al., and identical results are obtained with mathematically equivalent models.

In addition to likelihood ratio tests, Burnham and Anderson (1992) and Lebreton et al. (1992, p. 84) have shown that Akaike's information criterion (AIC) is a useful diagnostic for model selection. The AIC of a model is computed as

$$\text{AIC} = -2 \log_e(L) + 2n$$

in which n is the number of estimable parameters. Although the value of AIC decreases as a model's fit (L) improves, AIC increases with n to penalize complex models that overfit the data by including an excessive number of parameters. Therefore, minimization of AIC can be used to assist the selection of parsimonious models. Comparisons of AIC values and likelihood ratio tests often provide similar results in model selection problems (Lebreton et al. 1992, p. 111), but the former can be used also to compare nonhierarchical models.

Akaike's information criterion is particularly helpful for analyses that include comparisons of several alternative models, such as the backward elimination scheme described earlier for comparing reduced-parameter models. If the comparisons are made solely by likelihood ratio tests, the overall probability of incorrectly rejecting at least one of the null hypotheses tested can be much higher than the nominal α level (Lebreton et al. 1992, p. 111). An approximate upper bound on the overall Type I error probability of s separate likelihood ratio tests is $1 - (1 - \alpha)^s$, where α is the significance level of any one test (Agresti 1990, p. 213). Therefore, if several likelihood ratio tests are used in the backward elimination search for a parsimonious model, the overall risk of Type I error may be much higher than α. To minimize the inflation of overall Type I error in such cases, Lebreton et al. (1992, p. 111) recommended using a combination of AIC minimization and likelihood ratio testing for model selection. First, AIC values of the most biologically plausible models are computed to determine a smaller set of models with the lowest AIC values. Likelihood ratio tests are used then to conduct formal hypothesis tests with the AIC-selected models. This method of model selection is particularly helpful in cases where the choice of a low α value, say 0.05, for testing null hypotheses, say H_0: $\tau_g = 0$ $(g = 2, \ldots, m)$, produces tests of low statistical power due to low recovery rates (see paragraph below and Discussion). In these situations, one may want to accept a higher Type I error probability, say 0.1, to reduce the risk of falsely accepting H_0 and incorrectly concluding that $\tau_g = 0$ $(g = 2, \ldots, m)$ (Lebreton et al. 1992, p. 83).

During planning or evaluation of tag-recovery studies, analysts may need to ascertain how well temporal or group-specific differences in survival of predetermined magnitude can be detected. To do this, one needs to compute the statistical power of detecting significant differences in the goodness-of-fit of different survival models. Annual rates of survival in the null model, for example, may be constant, while those in the alternative model vary among groups. Given adequate sample sizes, the power function for comparing the goodness-of-fit of two multinomial models can be approximated with a noncentral chi-square distribution (Burnham et al. 1987, p. 214; Agresti 1990, p. 241). The noncentrality parameter for an individual comparison of two models is easily computed by using the expected numbers of recoveries under the alternative model as "data" for the estimators of the null model. The resulting chi-square goodness-of-fit test statistic of the null model is equivalent to the noncentrality parameter of the comparison. Statistical analysis software, such as SYSTAT or SAS, can compute power from the value of the noncentrality parameter, the significance level α, and the degrees of freedom associated with the test between the two models of interest.

TABLE 3. Recoveries of Atlantic striped bass tagged in New York (Hudson River stock) and in Maryland (Chesapeake Bay stock) during the 1988–1990 spring spawning seasons.

Year released	Number released	Year of recovery 1988	1989	1990
Recoveries of Hudson River stock				
1988	833	55	66	47
1989	691		58	50
1990	907			103
Recoveries of Chesapeake Bay stock				
1988	3288	146	97	129
1989	2904		97	158
1990	1494			91

An Example with Striped Bass

Since 1986, anadromous striped bass have been captured, tagged, and released at various locations along the east coast of the United States to help determine population-level mortality rates. The population comprises at least two distinct stocks: fish of Hudson River (New York) origin and fish of Chesapeake Bay origin. These stocks undergo extensive seasonal migrations and typically intermingle in nearshore areas of the Atlantic Ocean during summer, fall, and winter. Therefore, tagging of individual stocks normally occurs in the spring when the stocks occupy geographically distinct spawning areas.

To determine if survival rates of Hudson River and Chesapeake Bay stocks were significantly different during 1988–89, I analyzed recoveries of striped bass that were released during spring 1988–90 by the New York State Department of Environmental Conservation and by the Maryland Department of Natural Resources. Most recoveries of the tagged fish were reported by recreational and commercial fishermen who returned tags and information to the U.S. Fish and Wildlife Service in exchange for rewards.

Numbers of releases and recoveries of tagged Hudson River and Chesapeake Bay stocks are summarized in Table 3. Tests of various hypotheses about the effects of time (T) and stock (G) on rates of survival and recovery are summarized in Table 4. The fully parameterized model $(f_{T\cdot G}, S_{T\cdot G})$ fits the data fairly well ($P = 0.431$) and provides a reasonable starting point for hypothesis testing and model selection. A comparison of models (1) to (5) by likelihood ratio tests and AIC values indi-

TABLE 4. Modeling survival and recovery rates of different stocks of Atlantic striped bass. Summarized for each model are the number of estimable parameters (n), the negative of the log-likelihood ($-\log_e(L)$), Akaike's information criterion (AIC), and the significance probability of the model's chi-square goodness-of-fit (GOF). Models are compared by likelihood ratio tests to test various hypotheses about time (T) and stock (G) effects. See Table 2 for model notation.

Model	n	$-\log_e(L)$	AIC	GOF	Comparison by likelihood ratio test
(1) $(f_{T\cdot G}, S_{T\cdot G})$	10	38.10	96.19	0.431	
(2) $(f_{T\cdot G}, S_{T+G})$	9	39.32	96.65	0.247	Time–stock interaction in survival rates (2) vs. (1): $P = 0.117$
(3) $(f_{T\cdot G}, S_T)$	8	39.78	95.57	0.281	Stock effect on survival rates (3) vs. (2): $P = 0.337$
(4) $(f_{T\cdot G}, S_G)$	8	39.51	95.01	0.343	Time effect on survival rates (4) vs. (2): $P = 0.545$
(5) $(f_{T\cdot G}, S)$	7	39.97	93.93	0.366	Stock effect on survival rates (5) vs. (4): $P = 0.337$ Time effect on survival rates (5) vs. (3): $P = 0.545$
(6) (f_{T+G}, S)	5	43.63	97.25	0.079	Time–stock interaction in recovery rates (6) vs. (5): $P = 0.026$

cates that the effects of time, stock, and time–stock interactions on survival are not statistically significant, given the recovery rate model ($f_{T\cdot G}$). The likelihood ratio test between models (5) and (6) with $\alpha = 0.05$ indicates that the time–stock interaction in recovery rates is significant ($P = 0.026$). Therefore, model (5) with constant survival, $(f_{T\cdot G}, S)$, provides the best fit to the data with fewest parameters (and has the lowest AIC value).

Table 5 provides estimates of model parameters and Table 6 provides estimates of the year- and stock-specific rates of survival and recovery for the fully parameterized model $(f_{T\cdot G}, S_{T\cdot G})$ and for the reduced model $(f_{T\cdot G}, S)$. Coefficients of variation in estimates of survival range from 0.09 to 0.15 under model $(f_{T\cdot G}, S_{T\cdot G})$ whereas under model $(f_{T\cdot G}, S)$ the coefficient of variation of S is only 0.05. The improved precision is expected because model $(f_{T\cdot G}, S)$ contains fewer parameters than the more general model $(f_{T\cdot G}, S_{T\cdot G})$; however, the variance estimates of the parameters of the reduced model are correct only if the model is correct, in the sense that it approximates the true state of nature.

Discussion

The model developed here provides a statistical framework for examining time- and group-specific variation in survival and recovery rates. By selectively constraining the model's parameters, less important sources of variation can be eliminated to improve the precision of survival and recovery rate estimates without introducing important bias.

Survival (or recovery) rates of different groups of tagged animals previously were compared by analyzing each group's recoveries separately and forming linear contrasts of the estimates (Brownie et al. 1985; Sauer and Williams 1989). Separate analyses of each group's data, however, include estimation of unneeded parameters when some rates vary among groups and others do not. The result is an unnecessary loss of precision and a reduction in the statistical power of detecting differences among groups.

The method described here of testing hypotheses by stepwise evaluation of alternative submodels does not suffer from these

TABLE 5. Maximum likelihood estimates of parameters of models $(f_{T\cdot G}, S_{T\cdot G})$ and $(f_{T\cdot G}, S)$ applied to Hudson River and Chesapeake Bay stocks of striped bass. Standard errors of parameter estimates are shown in parentheses. Asterisks indicate parameter estimates that are significantly different from zero ($P < 0.05$).

Parameter	Model $(f_{T\cdot G}, S_{T\cdot G})$	Model $(f_{T\cdot G}, S)$
f_{11} (recovery rate of Hudson River stock in 1988)	0.066* (0.009)	0.066* (0.009)
α_2 (effect of 1989 on f_{11})	0.022 (0.013)	0.025* (0.012)
α_3 (effect of 1990 on f_{11})	0.048* (0.014)	0.036* (0.012)
β_2 (effect of Chesapeake stock on f_{11})	−0.022* (0.009)	−0.022* (0.009)
δ_{22} (effect of a 1989 – Chesapeake stock interaction on f_{11})	−0.031* (0.013)	−0.034* (0.013)
δ_{32} (effect of a 1990 – Chesapeake stock interaction on f_{11})	−0.031* (0.015)	−0.015* (0.012)
S_{11} (survival rate of Hudson River stock in 1988)	0.87* (0.11)	0.78* (0.04)
μ_2 (effect of 1989 on S_{11})	−0.26 (0.17)	
τ_2 (effect of Chesapeake stock on S_{11})	−0.09 (0.12)	
σ_{22} (effect of a 1989 – Chesapeake stock interaction on S_{11})	0.34 (0.22)	

TABLE 6. Estimates of year- and stock-specific survival and recovery rates computed from parameter estimates of models $(f_{T\cdot G}, S_{T\cdot G})$ and $(f_{T\cdot G}, S)$ shown in Table 5. Standard errors of rate estimates are shown in parentheses.

	Hudson River stock		Chesapeake Bay stock	
Year	Rate of recovery	Rate of survival	Rate of recovery	Rate of survival
Model $(f_{T\cdot G}, S_{T\cdot G})$				
1988	0.066 (0.009)	0.87 (0.11)	0.044 (0.004)	0.78 (0.07)
1989	0.087 (0.009)	0.60 (0.09)	0.035 (0.003)	0.86 (0.11)
1990	0.114 (0.011)		0.061 (0.006)	
Model $(f_{T\cdot G}, S)$				
1988	0.066 (0.009)	0.78 (0.04)	0.044 (0.004)	0.78 (0.04)
1989	0.092 (0.008)	0.78 (0.04)	0.035 (0.003)	0.78 (0.04)
1990	0.102 (0.008)		0.065 (0.005)	

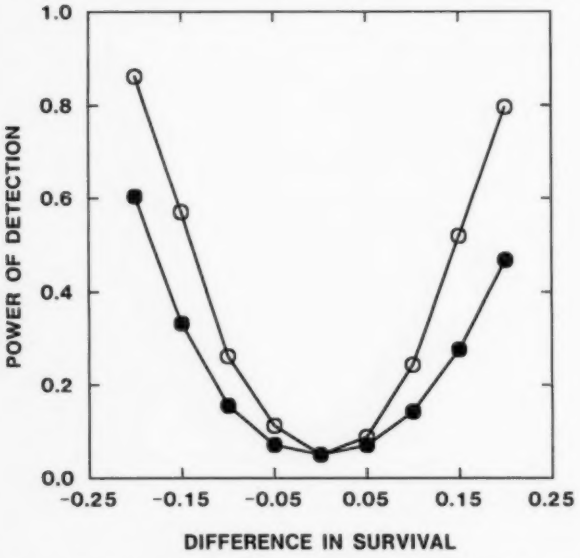

FIG. 1. Power curves for detecting hypothesized differences in survival of Hudson River and Chesapeake Bay stocks of striped bass. To compute power, nine sets of expected numbers of recoveries were computed by using the parameter estimates of model $(f_{T\cdot G}, S)$ in Table 5 and varying τ_g from -0.2 to 0.2 in steps of 0.05. Each set of expected recoveries was used as "data" and fit to model $(f_{T\cdot G}, S)$. The chi-square value of the resulting fit is the noncentrality parameter of the noncentral chi-square distribution used to calculate power with $\alpha = 0.05$ (see text). Filled symbols indicate power calculations with actual numbers of releases; open symbols indicated power calculations for a hypothesized fourfold increase in releases of Hudson River fish.

limitations. Model parameters may be constrained to account explicitly for similarities among groups, thereby providing more precise estimates and better chances of detecting differences in survival among groups. This does not guarantee, however, that the among-group differences will be easy to detect. The scope of inference is ultimately limited by the data. Consider, for example, the inability to detect significant differences in survival of the two stocks of striped bass (Table 4). The significance level of this test is 0.337, but what magnitude of difference could have been detected given the numbers of fish released and the rates of recovery during 1988–90? Also, if the number of striped bass released in the Hudson River had been increased fourfold to lessen the disparity in the numbers of fish released annually (Table 3), how much improved would have been the chances of detecting between-stock differences in survival? Questions of this sort may be explored by calculating statistical power. Figure 1 illustrates power curves for detecting hypothesized differences in survival of Hudson River and Chesapeake Bay stocks. The estimated survival of Chesapeake Bay fish is about 0.07 higher than that of Hudson River fish if the data are fit to model $(f_{T\cdot G}, S_G)$. Given the numbers of releases of both stocks during 1988–90, the power of detecting this difference is only 0.09 when testing at $\alpha = 0.05$. A fourfold increase in the annual releases of Hudson River fish increases the power to only 0.13; therefore, the chances of detecting a difference in survival rates of 0.07 are limited by the relatively low ($\leq$0.11) rates of recovery (Table 6). At these rates, differences in survival of the two striped bass stocks would need to have exceeded 0.20 to be detected at least 50% of the time.

In the statistical model developed here, survival and recovery rates are formulated as linear combinations of parameters that include the effects of time, group, and time–group interactions. I have shown that this formulation is equivalent to the time-dependent model described by Brownie et al. (1985) for analyzing unstratified populations. In addition, this formulation can be used to examine interesting, specialized, reduced-parameter versions of this more general model by further constraining the parameters in SURVIV (White 1983). For example, step changes in recovery rates associated with changes in fishing regulations can be modeled by constraining the effects of time on recovery rates to equal a common value prior to a change in regulations and to equal another value subsequently. Inclusion of such prior knowledge of the recovery process can improve the precision of the parameter estimates. Because the model developed here is easily extended to include the effects of multiple prerelease strata (e.g. stock and sex), many interesting reduced-parameter versions of the model can be explored.

The program SURVIV (White 1983) accommodates many different types of models, but SURVIV also possesses some limitations. Most notably, SURVIV is not designed to maximize likelihood functions subject to inequality constraints involving expressions of constants and two or more parameters. In SURVIV the possible types of constraints are limited to inequalities or equalities between two parameters or between a parameter and a constant. This prevents the estimation of reduced-parameter versions of certain models. For example, in the model developed here, a reduction in parameters is needed when the number of recovery years exceeds the number of release years and both survival and recovery rates are assumed to vary with time. This is true because the individual annual

rates of survival and recovery are not separately identifiable for the years that follow the last year of release (Brownie et al. 1985); only one additional parameter can be estimated for each of these years. To specify the correct reduction in the number of estimable parameters of the model developed here, each sum of parameters used to express the rate of survival during each of the years k through $l - 1$ must be constrained to equal unity. Software does exist to perform such constrained optimizations; however, the current version of SURVIV is not capable of doing so.

In the model developed here the identity function "links" (as used by Agresti (1990, p. 80) and Lebreton et al. (1992, p. 76)) the probabilities of survival and recovery to the linear combination of time and group effects parameters. Other link functions are possible, of course. The logit link function, for example, ensures that estimates of S and f remain within the interval (0,1) and is used in the program SURGE for analysis of capture–recapture data (Lebreton et al. 1992). The logit function does seem preferable to the linear probability model; however, the latter was selected mainly to avoid the limitations of SURVIV. In the DOS version of SURVIV the FORTRAN expressions for cell probabilities may not exceed 255 characters. This upper limit makes it difficult to implement the logit link model when the effects of several times and groups are modeled. More importantly, however, is SURVIV's limited ability to constrain the values of parameter estimates, as discussed earlier. Estimates of time or group effects on a logit scale can vary widely during iterative optimization when the estimated probabilities of S or f approach 0 or 1. Because tag recovery rates are usually low, convergence problems could hinder parameter estimation. By using SURVIV with a linear probability model, the estimattes of S_{11} and f_{11} can be constrained to the (0,1) interval. Initial guesses of the time and group effects parameters can be computed from the analytical solutions of model 1 of Brownie et al. (1985), and estimates of these parameters will not be as wide-ranging as they would be on a logit scale. However, if more versatile optimization software is used, the time and group effects parameters can be estimated on a logit scale.

The statistical models and testing approach developed here parallel the recent advances in capture–recapture methodologies (Lebreton et al. 1992). Capture–recapture models exist for examining the effects of prerelease strata, age or time dependence, and time covariates on survival and recapture rates. Advances in tag-recovery methodologies have not come as far. Models that include the effect of time covariates or variable-length recovery times have been developed by Conroy and Williams (1984) and implemented in a computer program called MULT (Conroy et al. 1989). Schwarz et al. (1988) have developed tag-recovery models to estimate the effects of post-release strata on estimates of survival or recovery. In this case the strata are determined at recovery. Examples with fish populations could include recoveries from different types of gear or from geographically distinct fisheries. These advances are important, but further work is needed. Addition of covariates, for example, would increase the versatility of tag-recovery models. The covariates might vary with recovery times (e.g. annual measurements of fishing effort) or they might vary among groups (e.g. differences in treatment levels in controlled experiments) or individuals (e.g. differences in "play" times of fish angled for tagging). Tests of hypotheses about survival often have low power due to low recovery rates. Further advances in tag-recovery models nonetheless offer some exciting prospects for large-scale experiments with tagged fish populations.

Acknowledgments

This work was simulated by discussions with D. R. Anderson, K. P. Burnham, and J. D. Lebreton during a workshop on statistical methodologies of survival estimation. I am grateful to them for developing the workshop and providing many insights about the analysis of capture–recapture and tag-recovery data. Thanks also go to A. Kahnle and K. Hattala (New York State Department of Environmental Conservation), S. Early, H. Hornick, A. Loftus, and C. McCollough (Maryland Department of Natural Resources), and B. Batky, J. Skjeveland, and C. Wooley (U.S. Fish and Wildlife Service). These people captured, tagged, and released the striped bass and assisted in various aspects of data collection. Finally I thank P. Rago and two anonymous referees for reviewing the manuscript and providing helpful comments. My work was supported in part by the Emergency Striped Bass Research Study.

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Localisation cytologique du cuivre et de quelques autres métaux dans la glande digestive de la seiche, *Sepia officinalis* L. (Mollusque Céphalopode)

Micheline Martoja

Institut océanographique, 195, rue Saint Jacques, 75005 Paris, France

et Christiane Marcaillou

Université Pierre et Marie Curie, Laboratoire d'histophysiologie fondamentale et appliquée, 12, rue Cuvier, 75005 Paris, France

Martoja, M. et C. Marcaillou. 1993. Localisation cytologique du cuivre et de quelques autres métaux dans la glande digestive de la seiche, *Sepia officinalis* L. (Mollusque Céphalopode). Can. J. Fish. Aquat. Sci. 50 : 542–550.

La glande digestive de la seiche, *Sepia officinalis* L., a été étudiée par méthodes histochimiques et microanalytiques. La majeure partie du Cu y est concentrée dans des sphérules élaborées par les cellules basales. Il y est accompagné d'Ag et de Zn. Du Fe existe également mais il est plus abondant dans les cellules digestives. Nos résultats montrent que les sphérules sont constituées de "metallothionein-like proteins". Le fait que les ligands des métaux soient des protéines a une incidence sur le plan écologique. Les métaux sont facilement assimilables et peuvent être transférés aux niveaux supérieurs de la chaîne trophique.

The cuttlefish, *Sepia officinalis* L., liver was investigated by histochemical and microanalytical methods. The greater part of the accumulated Cu is concentrated in spherulae which are elaborated by the basal cells. Cu is associated with both Ag and Zn. Fe is also found but the digestive cells are the most important site of Fe storage. Our data show that the spherulae are made of metallothionein-like proteins. The occurrence of these metal-binding proteins has ecological effects. The metals are easily assimilable and can be transferred to higher levels of the food chain.

Reçu le 11 mars 1992
Accepté le 18 septembre 1992
(JB434)

Received March 11, 1992
Accepted September 18, 1992

Les céphalopodes accumulent, dans les conditions naturelles, une importante quantité de Cu. Celui-ci est réparti de façon inégale entre les organes mais c'est toujours la glande digestive qui en contient le plus. Chez la seiche, *Sepia officinalis* L., elle détient 50 % du métal alors qu'elle ne représente que 6 % du poids de l'animal (Miramand *et al.* 1991), les teneurs moyennes, exprimées en poids sec, étant de l'ordre de 298 $\mu g \cdot g^{-1}$ pour les juvéniles et de 1 200 $\mu g \cdot g^{-1}$ pour les adultes (Wang Tai Si 1928; Schipp et Hevert 1978). Cependant, ces données quantitatives ne concordent pas avec les résultats de l'histochimie : certes, ce mode d'analyse décèle du Cu dans les cellules digestives mais l'intensité des réactions est très inférieure à ce que laissent prévoir les dosages (Ruth *et al.* 1988). Schipp et Hevert (1978) précisent même que "copper has not yet been localized on cytochemical basis". Par ailleurs, on sait que, chez divers céphalopodes, le Cu peut être associé à d'autres métaux et en particulier à du Zn, du Cd et de l'Ag (Martin et Flegal 1975; Finger et Smith 1987), mais le mode d'association est inconnu. Ces considérations nous ont conduites à reprendre l'examen de la glande digestive de la seiche, par diverses méthodes histologiques et microanalytiques, dans le but de localiser des accumulations de Cu plus importantes que celles des cellules digestives et, le cas échéant, de déterminer les ligands du métal.

Matériel et méthodes

Les glandes digestives et quelques glandes branchiales ont été prélevées sur des seiches adultes, récoltées au cours de divers séjours effectués sur la côte méditerranéenne (Laboratoire Arago, Banyuls-sur-mer).

Les animaux ont été sacrifiés par décapitation. Certaines pièces ont été fixées par les mélanges de Bouin ou de Carnoy pendant 12 à 24 h, puis conservées dans l'éthanol ou incluses dans la paraffine. Les coupes, de 5 µm d'épaisseur, ont été traitées par les méthodes suivantes : trichrome de Prenant–Gabe et coloration au rouge solide-picro-indigocarmin (examen topographique); coloration au bleu de méthylène (détection des composés basophiles); réaction à l'acide periodique-Schiff (APS) (détection des composés glucidiques); tétrazoréaction de Danielli (détection des protéines); réaction de Bennett au red sulfhydril reagent (RSR) (détection spécifique des groupes SH); réaction au ferricyanure ferrique de Schmorl (mise en évidence du caractère réducteur); réaction à l'hexaméthylène tétramine d'Ag de Gomori (détection de l'acide urique, des urates et des phénols); réaction de substitution par l'Ag de von Kossa (détection des phosphates et des carbonates); réaction de Bunting à l'acide sulfurique (détection spécifique des carbonates); coloration par la laque d'alizarine de Dahl et McGee–Russell (détection du Ca, du Mg, du Sr et de l'Al); réaction de Kashiwa au glyoxal-bis 2-hydroxyanil (GBHA) (détection spécifique du Ca); réaction à l'acide rubéanique d'Okamoto et coloration par l'hématoxyline de MacCallum (détection du Cu); réaction de Tirmann et Schmeltzer au ferricyanure de potassium (détection du Fe bivalent et du Cu monovalent); réaction de Perls au ferrocyanure de potassium (détection du Fe trivalent et du Cu biva-

lent) (voir Langeron 1949, Martoja et Martoja 1967 et Pearse 1972 pour le détail des techniques).

Pour la microscopie électronique par balayage, les pièces ont été fracturées dans l'azote liquide et déshydratées par l'éthanol absolu. Après dessiccation par la technique du point critique, les échantillons ont été métallisés par évaporation d'or–palladium.

Pour la microscopie électronique à transmission, les pièces ont été fixées pendant 90 min, à 4 °C, par le glutaraldéhyde à 3 % dans un tampon cacodylate de sodium 0,2 M additionné de saccharose 0,35 M (pH 7,1), post-fixées par le tetroxyde d'osmium à 2 % et incluses dans l'épon–araldite. Le contraste des coupes a été renforcé par l'acétate d'uranyle et le citrate de plomb.

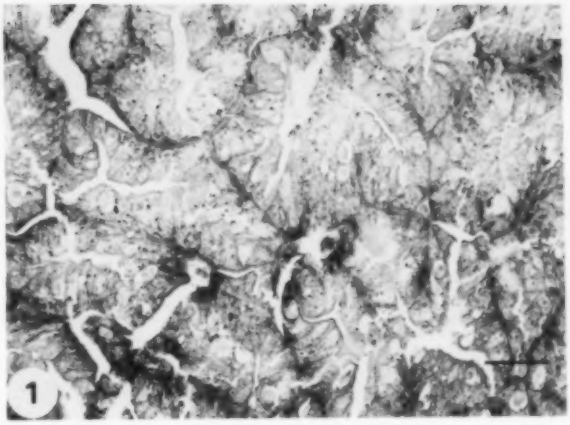
La microanalyse a été effectuées par deux méthodes : 1) spectrographie des rayons X, au moyen d'une microsonde électronique équipée de spectromètres dispersifs en longueur d'onde (Cameca MS 46), avec une tension d'accélération des électrons de 15 kV, un courant d'échantillon de 40 nA et un diamètre de sonde de 1 μm. Les coupes ont été étalées sur support de mylar carboné et déparaffinées. Les éléments P et S ont été recherchés avec le cristal KAP (phtalate acide de potassium), Cu et Zn avec le cristal LiF (fluorure de lithium) et Ag et Cd avec le cristal PET (penta-érythritol); 2) émission ionique secondaire au moyen d'un analyseur Cameca SMI 300, sur coupes à la paraffine de 7 μm, étalées sur support d'or poli et déparaffinées, soumises aux conditions standard d'analyse en basse résolution en masse (bombardement : O_2^+, 7 μA, 10 kV, diaphragme de contraste 200 μm, diamètre de champ imagé 250 μm, grandissement direct 110 environ, résolution en masse du spectromètre $m/\nabla m = 300$).

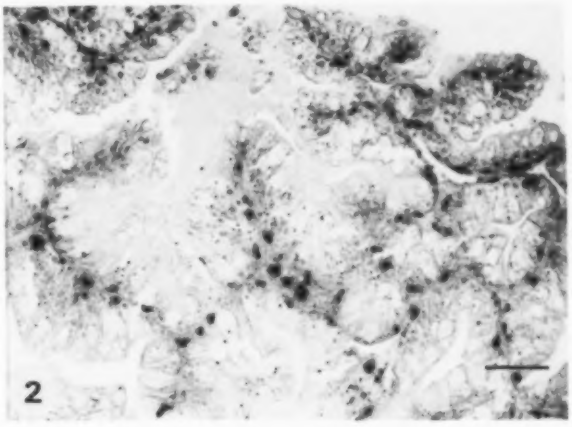
Résultats

Caractères des cellules basales

Le Cu a été mis en évidence dans l'hémocyanine ainsi que dans de minuscules grains localisés dans les cellules digestives et parfois dans la lumière de la glande. Toutefois, les concentrations les plus importantes ont été décelées dans des cellules triangulaires situées à la base de l'épithélium, entre les cellules digestives. Suivant la terminologie de Cuénot (1907), nous les nommons cellules basales. Dès l'examen topographique, elles se distinguent des cellules digestives par leur basophilie. Après fixation par le mélange de Bouin ou le glutaraldéhyde, leur cytoplasme est spongieux. Après fixation par le mélange de Carnoy, les examens en microscopie photonique ou en microscopie par balayage montrent que cet aspect est dû à la dissolution de sphérules qui peuvent atteindre 5 μm et dont le nombre varie considérablement d'un individu à l'autre (fig. 1 à 4).

À l'échelle ultrastructurale, les cellules basales se caractérisent par la coexistence d'un reticulum endoplasmique granulaire très développé et de plages circulaires, transparentes aux électrons, correspondant aux sphérules. Le reticulum est disposé en nappes parallèles à la lame basale, plus nombreuses au voisinage de celle-ci qu'à l'apex cellulaire (fig. 5 et 6). Les citernes sont peu dilatées et denses. Les grains de sécrétion renferment un produit granuleux ne présentant aucune organisation particulière qui permette de l'identifier (fig. 7). Les territoires hyaloplasmiques, séquestrés entre les nappes de reticulum ont, au départ, une forme irrégulière. Ils acquièrent progressivement leur transparence tandis que leur volume augmente grâce à l'apport des vésicules qui restent d'abord





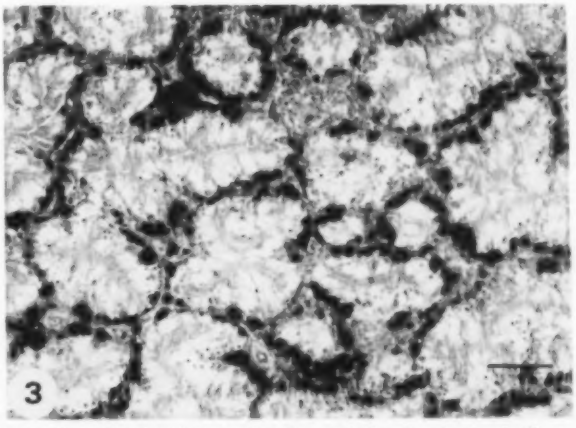


FIG. 1 à 3. Variabilité du nombre des sphérules dans la glande digestive. Selon les individus, les sphérules, qui apparaissent en noir à la périphérie des tubes, peuvent être absentes (fig. 1), assez rares (fig. 2) ou extrêmement abondantes (fig. 3) (fixateur de Carnoy; réaction de Schmorl, rouge solide; l'échelle représente 60 μm).

cantonnées en périphérie (fig. 8). Peu à peu, leur contour se régularise puis ils fusionnent entre eux par groupes de trois ou quatre et leur morphologie se rapproche de plus en plus de celles des sphérules. À maturité, leur contenu est floconneux et il s'y

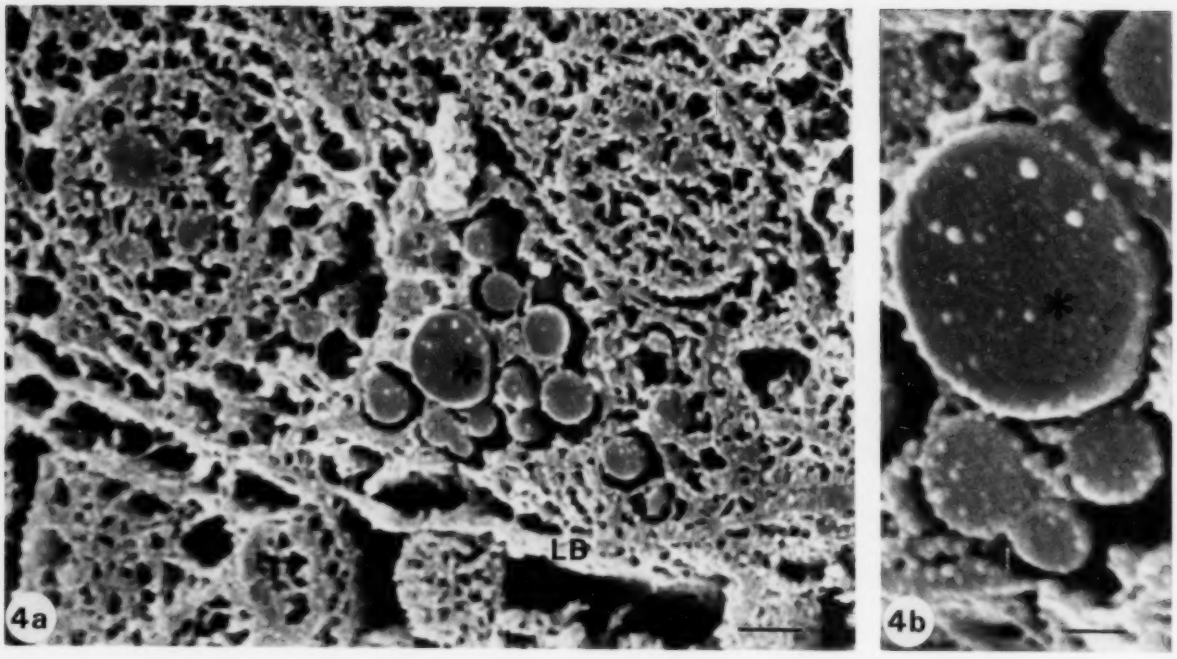


FIG. 4. Aspect d'une cellule basale en microscopie électronique à balayage. Le contenu des sphérules (astérisques), parfaitement conservé (fig. 4a), a un aspect granuleux et hétérogène (fig. 4b). (Fixateur de Carnoy; les échelles représentent respectivement 1 et 0,33 μm; LB : lame basale.)

ajoute parfois de petits amas plus opaques. Aucune image de pinocytose n'a été observée.

Au voisinage de l'hémolymphe, les espaces intercellulaires contiennent des molécules d'hémocyanine tout à fait identifiables. Quel que soit le sens du transit, il est donc évident que le pigment respiratoire traverse la lame basale. Cette dernière est ponctuée d'une rangée régulière de grains opaques (fig. 9). D'éventuels rapports de filiation entre ces grains et le pigment ne peuvent être envisagés qu'à titre d'hypothèse. En effet, les deux structures sont contiguës mais les grains n'ont pas la configuration typique de l'hémocyanine et aucune forme de transition n'a été observée. L'hémocyanine est très abondante entre les tubes de la glande digestive et les capillaires. Les molécules sont le plus souvent agencées en amas cristallins (fig. 10). Exceptionnellement, ceux-ci sont entourés d'une membrane (revoir fig. 5). Les espaces intertubulaires renferment aussi des mitochondries et des noyaux isolés qui, d'après leurs caractères, ne peuvent provenir de fibrocytes. Leur origine n'a pas été déterminée.

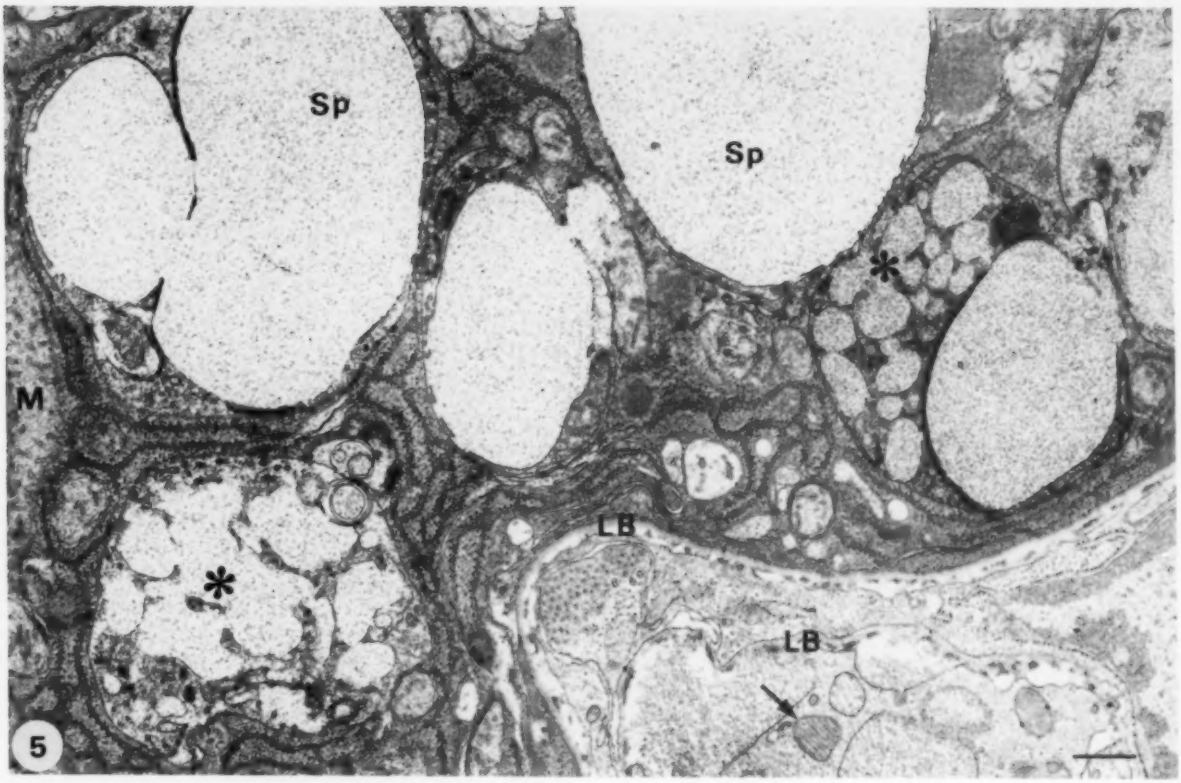
Caractères des sphérules

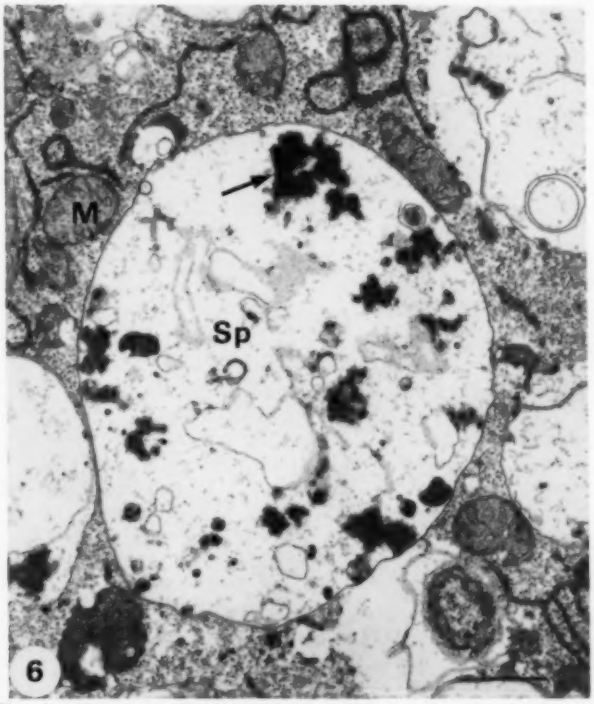
En raison de leur hydrosolubilité, l'étude microanalytique et histochimique des sphérules a été réalisée exclusivement sur matériel fixé par le mélange de Carnoy.

La microanalyse X montre une exceptionnelle richesse en Cu. A titre indicatif, les valeurs extrêmes, exprimées en coups comptés pendant 20 s, y sont de 1 200 à 4 800 et seulement de 160 à 300 pour les inclusions des cellules digestives, de 50–150 pour l'hémocyanine et de 30 à 40 pour les cellules des glandes branchiales. Le second élément majeur est le soufre. Le rapport des intensités Cu/S va de 0,7 à 3,0 selon les sphérules. Les autres éléments détectés sont, dans l'ordre décroissant : Zn > Ag et Ca > Mg > Sr (fig. 11 et 12). La recherche des éléments K, Na et P est restée négative. Le Cd n'a pas été décelé même en analyse ionique. Quelques sphérules contiennent du Fe mais la majeure partie ainsi qu'une certaine quantité de Zn sont localisées dans les cellules digestives.

Les sphérules sont solubles dans les acides minéraux (HCl 1 M, H_2SO_4 0,5 M, H_5IO_6, pH 1,4), les bases (NaOH 1 M ou NH_4OH 1 M), KCN, Li_2CO_3, les tampons glycine–HCl (pH 2,3) et véronal–HCl (pH 8,5). Elles sont lentement solubles dans une solution aqueuse d'acide acétique (CH_3CO_2H, pH 2,3) : un traitement de 15 min ne les altère pas; après 4 h, leur nombre a diminué et après 18 h, toutes ont disparu. Le soufre et les métaux Cu, Zn et Ag sont éliminés de façon progressive. En 18 h, le chlorure de cadmium, $CdCl_2$ à 1 %, déplace la totalité du Zn. Dans le même laps de temps, le bichlorure de mercure, $HgCl_2$ à 1 %, déplace 50 % du cuivre et 80 à 90 % du Zn. Dans les deux cas, la quantité de soufre reste inchangée. Les sphérules sont insolubles dans l'eau distillée (pH 5,5), l'acide picrique en solution aqueuse ou alcoolique, la pipérazine, le glycérol et le tampon borate (pH 8,5).

L'examen de coupes non colorées permet de distinguer, dans les sphérules, une fine coque et un coeur plus volumineux pourvu lui-même de quelques inclusions. La coque est naturellement noire tandis que le coeur est vert. Toutes les réactions et colorations signalétiques du Cu sont positives. L'hématoxyline aqueuse colore l'ensemble de la sphérule en bleu pâle et les inclusions du coeur en bleu vif. La réaction à l'acide rubéanique est intense (fig. 13); l'exposition aux vapeurs d'acide chlorhydrique, préconisée pour le démasquage du métal, ne la renforce pas mais, au contraire, l'abolit en dissolvant les sphérules. La réaction de Tirmann et Schmeltzer révèle du Cu bivalent coloré en brun rouge, surtout dans la coque et dans les inclusions du coeur. La réaction de Perls révèle du Cu mono-





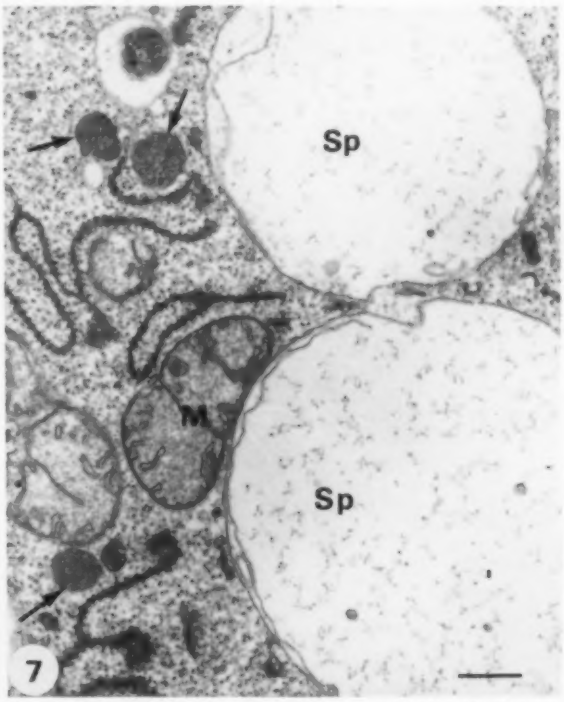


FIG. 5. à 7. Aspect ultrastructural des cellules basales. Fig. 5. Les plages du reticulum endoplasmique granulaire entourent de volumineuses sphérules (Sp) dont le contenu est dissous. On observe la formation de sphérules (astérisques) par la fusion de plusieurs territoires. Dans l'espace intertubulaire, une vésicule contient de l'hémocyanine (flèche) (l'échelle représente 0,5 μm). Fig. 6. Dans cette cellule, des amas (flèche) persistent dans les sphérules (l'échelle représente 0,62 μm). Fig. 7. Les grains de sécrétion ont un contenu granuleux (flèche) (l'échelle représente 0,45 μm). (LB : lame basale, M : mitochondrie.)

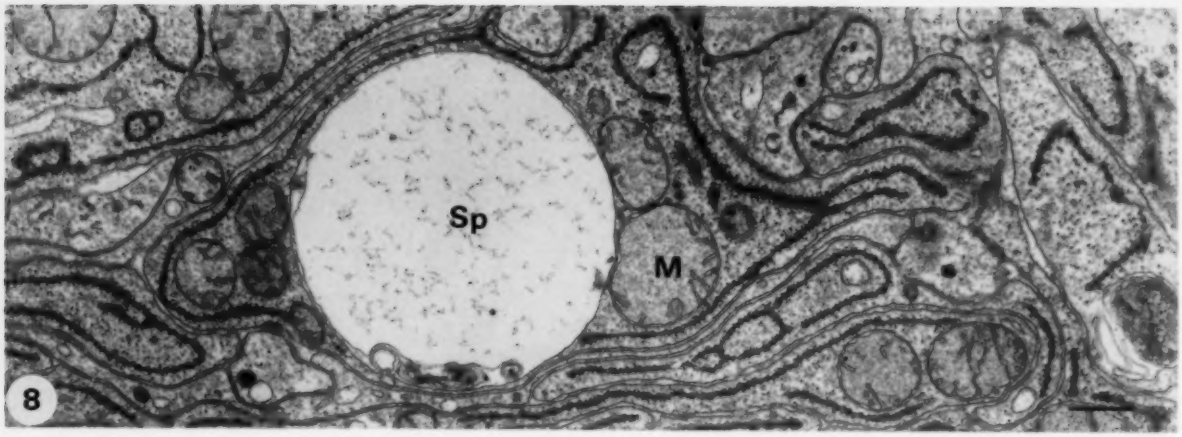


FIG. 8. Aspect ultrastructural d'une sphérule (Sp) montrant de petites vésicules accolées à la membrane limitante (l'échelle représente 0,57 μm; M : mitochondrie).

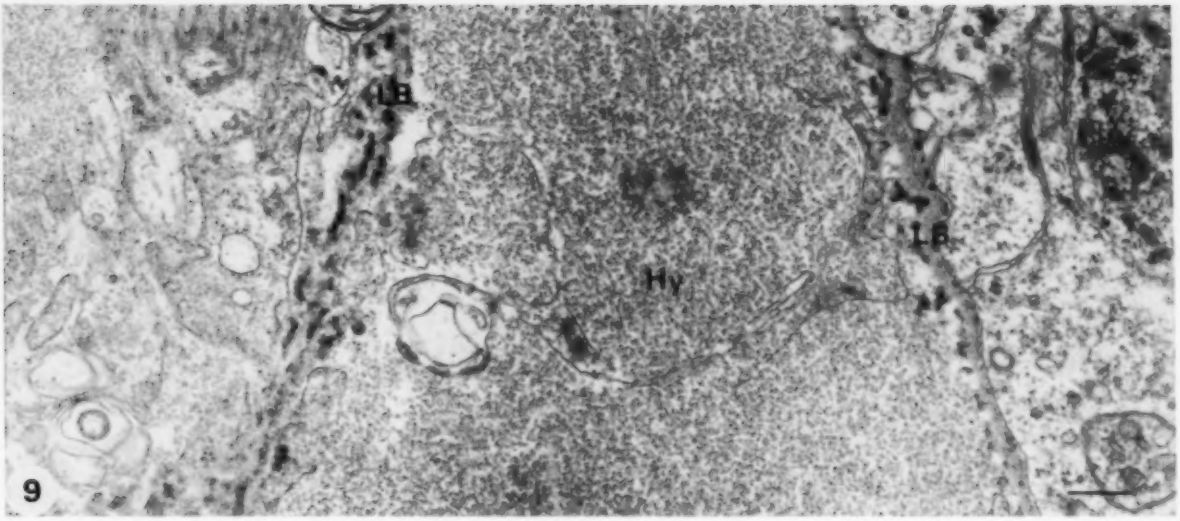
valent coloré de la même façon et, dans certaines sphérules, du Fe trivalent coloré en bleu.

La solubilité des sphérules limite le nombre des autres réactions histochimiques applicables. Ainsi, la réaction à l'APS est inutilisable. Une faible quantité de protéines est identifiable par la tétrazoréaction, dans les sphérules les plus petites. La détection spécifique des groupes SH par la méthode au RSR pratiquée en milieu anhydre (RSR en solution dans la diméthylformamide) est négative. En milieu acide, les sphérules présentent un caractère réducteur très prononcé, sans que celui-ci soit dû à de l'acide urique, à des urates ou à des polyphénols, la réaction de Gomori étant négative. L'absence d'urates coïncide avec le manque de Na et de K constaté en microanalyse. La réaction de Schmorl est positive dès les premières minutes qui suivent le contact avec le réactif (revoir fig. 2 et 3). Lorsque son déroulement est contrôlé par microanalyse X, il apparaît que 50 % du Cu et 80 % du Zn sont éliminés en 4 min; au bout de 20 min, 75 % du Cu ont disparu. Pendant ce même temps, la quantité de soufre ne varie pas. Il y a accord entre la microanalyse et la réaction au GBHA en ce qui concerne la faible quantité de Ca et c'est aux trois métaux alcalino-terreux, Ca, Mg et Sr, qu'il faut imputer la coloration par la laque d'alizarine. Le résultat négatif de la méthode de Bunting exclut la présence de carbonates et l'absence de P exclut celle de phosphates. Néanmoins, la réaction de Von Kossa est fortement positive si le traitement au nitrate d'argent est suivi d'une réduction par un révélateur photographique (fig. 14). S'il est accompagné d'une réduction par la lumière, la réaction est plus faible (fig. 15). La microanalyse montre que, en 20 min, 60 à 70 % du Cu naturel sont remplacés par l'Ag du réactif.

Discussion

Le but de ce travail était de déceler, dans la glande digestive de la seiche, des accumulations de Cu capables de rendre compte de sa teneur en métal. L'existence des cellules basales et de leurs sphérules nous semble résoudre le problème. Nos observations relatives à la présence de ces cellules sont en accord avec celles de Vigelius (1881), Frenzel (1886), Enriques (1902) et Cuénot (1907). Elles diffèrent de celles des auteurs actuels qui, ou bien ne mentionnent pas les cellules basales ou bien nient leur existence chez la seiche (voir Boucaud-Camou et Boucher-Rodoni 1983 pour la bibliographie). L'échec de leur mise en évidence s'explique, selon nous, par l'emploi de fixateurs inadaptés, tel le glutaraldéhyde qui dissout les sphérules et/ou par l'examen d'animaux juvéniles qui n'accumulent que peu de Cu par rapport aux adultes comme le montrent les valeurs de Schipp et Hevert (1978) et de Wang Tai Si (1928).

Le Cu des sphérules n'est pas engagé dans un composé simple puisque, dans un tel composé, le rapport Cu/S serait constant alors qu'il varie de façon continue dans une proportion de 1 à 4. La coexistence de Cu, de Zn et d'Ag ainsi que la forte teneur en soufre suggèrent que ces métaux sont complexés par une "métallothionéin-like protein" (MTLP). La conservation du ligand par les traitements histologiques anhydres laisse supposer, en outre, qu'il s'agit de polymères. Plusieurs arguments appuient cette hypothèse. La labilité des sphérules en milieu acide et leur stabilité en milieu neutre sont des caractères de polymères de MTLP. Elles expliquent les particularités du déroulement des réactions au ferricyanure ferrique et au RSR. La réduction du ferricyanure ferrique n'a lieu que si l'oxydation des groupes SH en groupe S–S est possible. Or, le Cu est très vite éliminé en raison du pH acide (2,3 à 4), ce qui libère les groupes SH. Il s'ensuit la réduction immédiate du réactif et la formation de ponts disulfures qui stabilisent l'apoprotéine. Le soufre reste décelable en microanalyse. Des ponts disulfures naturels existent d'ailleurs dans les polymères de métallothionéines. Le caractère négatif de la méthode au RSR démontre que tous les groupes SH sont complexés et, d'après la microanalyse, les métaux sont effectivement conservés pendant la réaction. Si on tente de les éliminer par un traitement acide prolongé, la recherche du soufre à la microsonde devient négative parce que l'apoprotéine et ses groupes SH sont eux-mêmes éliminés. Autre caractère essentiel des métallothionéines, les métaux les plus lourds déplacent ceux qui les précèdent. Or, dans les sphérules, le Hg déplace le cuivre (l'affinité des métallothionéines pour le Hg est utilisée pour leur dosage, Piotrowski *et al.* 1973). Cette aptitude explique également la substitution du Cu par l'Ag, lors de la réaction de Von Kossa. Si l'on restreint cette méthode à son but initial qui est la mise en évidence de carbonates et de phosphates alcalino-terreux, le résultat est faussement positif. La réduction en Ag-métal de l'Ag^+ substitué exige un agent énergique et cette difficulté de le réduire prouve que le résultat n'est pas dû à une liaison ionique mais



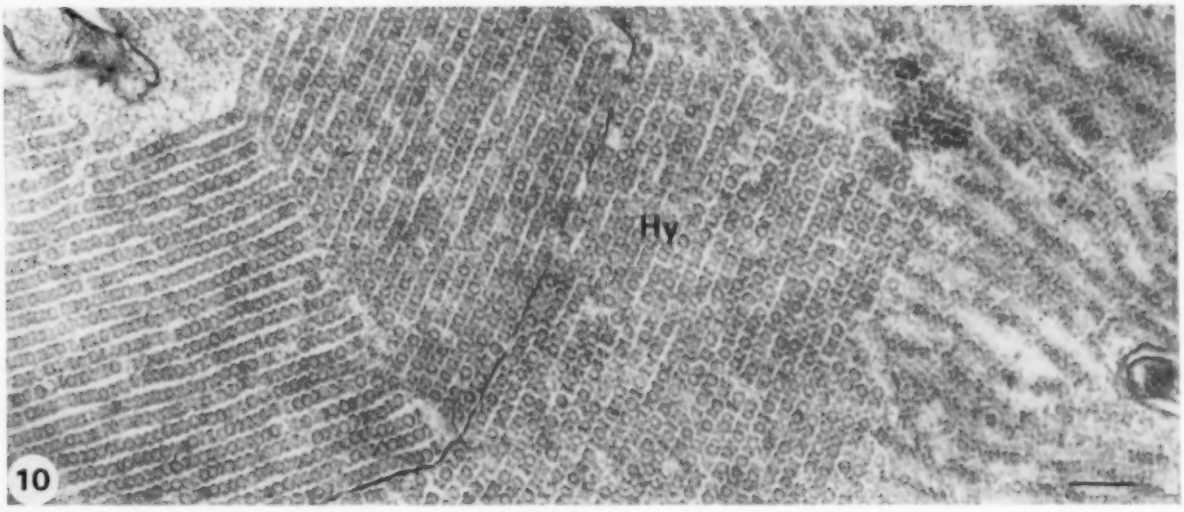
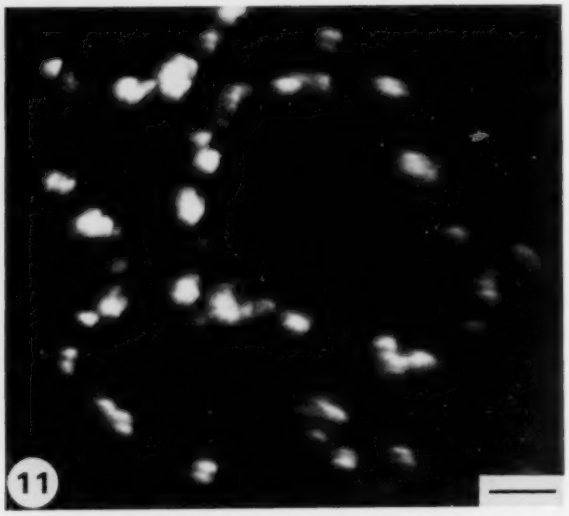


FIG. 9 et 10. Aspect ultrastructural d'un espace intertubulaire contenant de l'hémocyanine (Hy) dont les molécules sont éparses (fig. 9) ou organisées en réseau cristallin (fig. 10) (les échelles représentent respectivement 0,31 et 0,12 μm; LB : lame basale contenant des grains opaques).

à une liaison de co-valence. Ceci est encore en faveur de la présence de MTLP puisque leur affinité pour l'Ag résulte d'une complexation du métal avec les groupes SH, caractère lui-aussi utilisé pour leur dosage (Kägi et Vallee 1961). La conservation, sur pièces fixées, de la MTLP substituée par Ag s'explique par l'emploi d'un réactif à pH neutre et non par la formation de ponts intermoléculaires comme dans la réaction au ferricyanure ferrique.

Outre les métaux caractéristiques des MTLP, les sphérules contiennent du Fe, du Ca, du Mg et du Sr. Cette particularité a déjà été constatée chez des vertébrés (Kägi et Vallee 1961) et ne va pas à l'encontre de notre hypothèse. L'absence de phosphore et les images ultrastructurales démontrent que le Fe n'est pas engagé dans un composé de type hémosidérine, comme il l'est dans les cellules digestives (Boucaud-Camou 1973). Mais Fe^{+3}, métal de la classe A, a également des propriétés de la classe B. Il peut être lié à des acides aminés (voir Nieboer et Richardson 1980) et donc à des MTLP ou à d'autres protéines. Par ailleurs, le fait qu'aucun anion minéral ne corresponde aux alcalino-terreux et que, par conséquent, ceux-ci ne forment pas de sels minéraux est un argument en faveur de la présence de polymères. Il est connu, en effet, que les cations bivalents jouent le rôle de stabilisateurs d'édifices moléculaires en formant des ponts entre les molécules.

Il y a une concordance parfaite entre nos déductions et les résultats biochimiques, sur au moins deux points. D'une part, les variations du nombre des sphérules et de la quantité de métaux dans chacune d'elles rendent compte des écarts individuels qui apparaissent lors des dosages (Martin et Flegal 1975; Schipp et Hevert 1978; Finger et Smith 1987; Miramand *et al.* 1991). D'autre part, la spéciation chimique du Cu est la même que dans la glande digestive de *Nototodarus gouldi* où le Cu est associé à trois ligands (Finger et Smith 1987). Le premier (PM > 70 000) représente l'hémocyanine et les enzymes. Le second (PM = 11 500) correspond à des MTLP et le troisième (PM = 18 000) à un polymère du précédent. Selon nos obser-



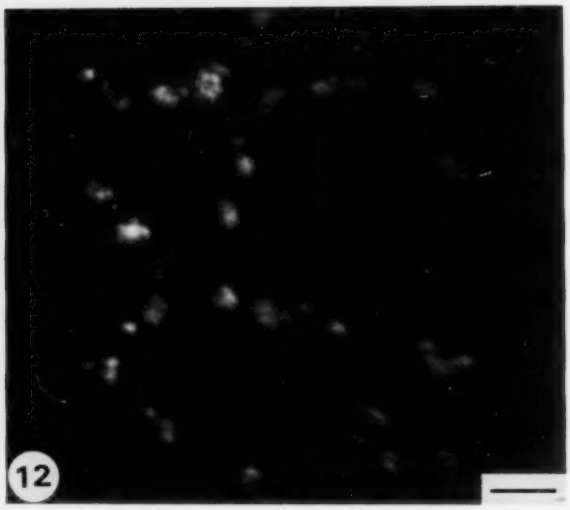


FIG. 11. et 12. Répartition du Cu (fig. 11) et de l'Ag (fig. 12) dans un tube de la glande digestive : remarquer la superposition rigoureuse des deux images d'émission ionique (détails techniques dans le texte; l'échelle représente 20 μm).

vations, ces deux derniers constituent les sphérules et le polymère, considéré comme un artefact par Finger et Smith, est bien un composé naturel. En ce qui concerne le Cd et le Zn, il est possible que l'absence de parallélisme entre la seiche et *Nototodarus* ne soit qu'un effet de la variabilité individuelle. Ainsi, les sphérules pourraient contenir du Cd sans que sa teneur ait atteint le seuil minimum de détection, de même qu'il n'a été trouvé, dans des MTLP, que chez les *Nototodarus* qui en étaient les plus riches. On pourrait expliquer de la même façon que les sphérules contiennent du Zn lié à des MTLP alors que des Zn-thionéines manquent chez les *Nototodarus* examinés. Enfin, les ligands de l'argent ne semblent pas avoir été déterminés chez d'autres céphalopodes mais ce que l'on sait de ses affinités chimiques laissent penser qu'il est lié à des protéines.

La synthèse de MTLP, qui est attestée par l'ultrastructure des cellules basales, pose le problème des homologies et du rôle de ces cellules. À notre connaissance, des MTLP localisées

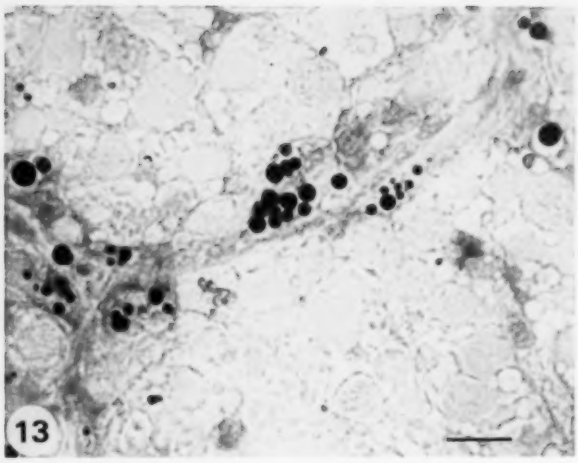
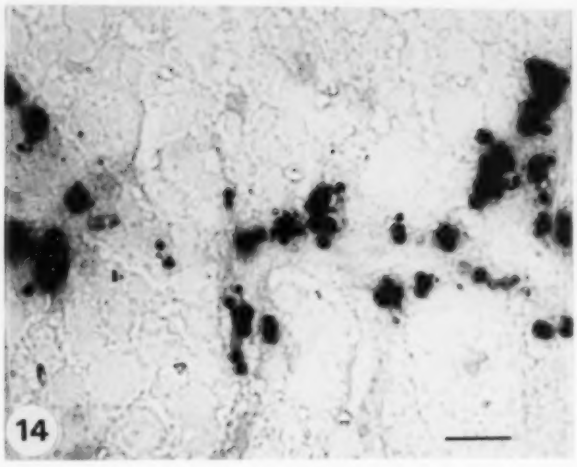


FIG. 13. Détection histochimique du Cu dans les sphérules des cellules basales (fixateur de Carnoy; réaction à l'acide rubéanique, rouge solide; l'échelle représente 12 μm).

ailleurs que dans des lysosomes, n'ont été mises en évidence, par méthode histologique, que dans les amoebocytes d'huîtres contaminées expérimentalement (Martoja et Martin 1985) et dans les coelomocytes d'un ver marin (Dhainaut-Courtois *et al.* 1987). Les cellules basales n'ont évidemment que peu de rapport avec ces types cellulaires. Au contraire, par leur forme, leur emplacement et certains caractères cytologiques, elles sont indiscutablement homologues de celles du nautile qui, selon Ruth *et al.* (1988), élaborent l'hémocyanine. L'hypothèse ne peut être retenue pour la seiche : il n'y a pas trace d'hémocyanine dans les cavités du reticulum endoplasmique et, de surcroît, il est prouvé que la synthèse a lieu dans les glandes branchiales (Dilly et Messenger 1972; Schipp *et al.* 1973; Muzzii 1981). Les cellules basales accumulent du cuivre comme les cellules à pores des gastéropodes (Sminia 1972) mais c'est là leur seul point commun. Dans les cellules à pores, le Cu issu de la dégradation de l'hémocyanine s'accumule dans des lysosomes, sous forme de sulfure dont la quantité progresse avec l'âge. Il en résulte que les plus vieux individus sont les plus riches en Cu (Martoja et Thiriot-Quiévreux 1980; Martoja *et al.* 1980; Bouquegneau et Martoja 1982). Au contraire, les cellules basales n'ont pas les caractères de cellules impliquées dans le catabolisme et le Cu est immobilisé dans un composé organique labile. Il est sans doute réutilisé ce qui expliquerait que, à l'inverse des gastéropodes, la concentration en Cu reste constante ou décroît quand le poids de l'animal augmente (Marks 1938; Finger et Smith 1987). La synthèse et la dégradation du pigment respiratoire avec fossilisation dans des lysosomes étant exclues, l'hypothèse la plus plausible est celle d'une régulation du Cu sanguin faisant appel à des MTLP. À l'exception du Fe, mieux représenté dans les cellules digestives, la régulation intéresserait également les autres métaux accompagnant le Cu. En effet, chez les invertébrés marins, les MTLP jouent un rôle primordial à la fois dans le métabolisme normal et dans la détoxication de certains métaux (voir Roesijadi 1981; Bouquegneau *et al.* 1984).

En conclusion, les sphérules des cellules basales de la glande digestive des seiches examinées contiennent quatre des cinqs métaux susceptibles de se concentrer dans la glande digestive, seul le Cd n'ayant pas été décelé. Le Cu, le Zn et le Fe existent dans d'autres structures mais les deux premiers sont beaucoup



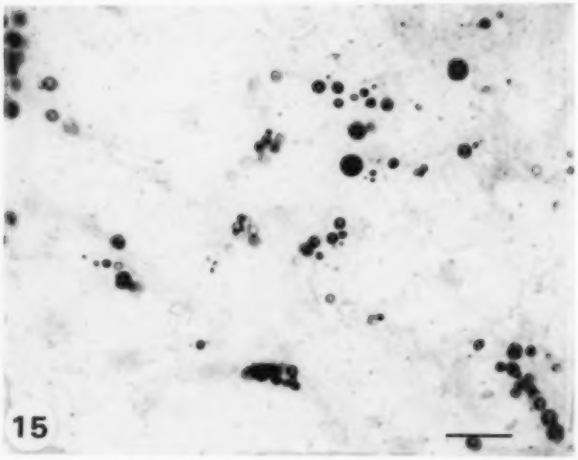


FIG. 14 et 15. Résultats du traitement par le nitrate d'argent suivi d'une réduction par un révélateur photographique (fig. 14) ou par la lumière (fig. 15). La différence d'intensité traduit la difficulté de réduction du composé substitué. (Fixateur de Carnoy; réaction de von Kossa, rouge solide; l'échelle représente 12 µm.)

plus abondants dans les sphérules au contraire du Fe qui est plus concentré dans les cellules digestives. Ils sont liés à un composé chimique ayant les caractères de MTLP. Le fait que les métaux soient associés à des protéines n'est pas sans conséquence sur la chaîne trophique, où les céphalopodes représentent un maillon extrêmement important. En effet, ces protéines sont fragmentées par digestion enzymatique et les fragments sont assimilés par les organes absorbants du consommateur. Le complexe cystéine-métal, en particulier, est très facilement assimilable. En outre, lorsque la digestion se fait à pH très bas, les métaux sont libérés de leurs ligands. Or, cette situation existe chez tous les vertébrés et notamment, chez les mammifères et les oiseaux marins qui sont les principaux prédateurs des céphalopodes (Mangold 1989). On peut donc affirmer que les cellules basales du foie constituent pour eux une importante source d'oligoéléments qu'ils soient essentiels, comme le Zn et surtout le Cu, ou toxiques, comme l'Ag et le Cd. Au contraire, l'association métal–protéines est sans danger pour l'homme, dans la mesure où il ne consomme que le muscle. Cette inocuité est intéressante quand on sait que les céphalopodes font l'objet d'une exploitation commerciale intensive et que 40 000 tonnes de seiches, *Sepia officinalis* et *S. esculenta*, sont pêchées chaque année au Japon et sur les côtes africaines (Worms 1989).

Remerciements

Nous remercions le Dr von Boletzky qui nous a procuré les animaux utilisés pour l'examen en microscopie électronique, Mmes Guillaumin et Saihi qui nous ont aidées pour la microscopie à balayage et l'analyse ionique, ainsi que le service commun de microscopie (CNRS-Paris VI) et le laboratoire de biologie des invertébrés marins du museum d'histoire naturelle où nous avons effectué l'étude ultrastructurale.

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Reproductive Biology of Atlantic Halibut (*Hippoglossus hippoglossus*) in Canadian Waters

John D. Neilson[1]

Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada[2]

John F. Kearney[3]

Maritime Fishermen's Union, Yarmouth, N.S. B5A 4B2, Canada

and Peter Perley and Harry Sampson

Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada

Neilson, J. D., J. A. Kearney, P. Perley, and H. Sampson. 1993. Reproductive biology of Atlantic halibut (*Hippoglossus hippoglossus*) in Canadian waters. Can. J. Fish. Aquat. Sci. 50: 551–563.

Through examination of commercial fishery samples, surveys conducted from research vessels, and fishery observer programs, the reproductive biology of Atlantic halibut (*Hippoglossus hippoglossus*) in the Canadian North Atlantic is described. Using data derived from conventional techniques including histology and visual assessment of maturity, and a more novel approach using image analyses equipment, halibut were found to be group-synchronous spawners whose spawning activity peaks in the November–December period, considerably earlier than was previously thought. Our analyses also allowed us to comment on the life history of Atlantic halibut in Canadian waters. The comparative absence of spawners in the continental shelf/slope areas may mean that halibut spawn in comparatively deep water, consistent with the habits of the Pacific member of the genus, *H. stenolepis*. Immature fish typically occurred on the southwestern Scotian Shelf but rarely off Newfoundland, supporting the view that the former area is an important rearing area for immature halibut.

La biologie de la production du flétan de l'Atlantique (*Hippoglossus hippoglossus*) dans la partie canadienne de l'Atlantique Nord est décrite à partir de l'examen d'échantillons de pêches commerciales, de relevés obtenus par des navires de recherche et par des programmes d'observateurs des pêches. Les résultats obtenus avec des techniques classiques comme l'histologie et l'évaluation de la maturité par l'examen direct, ainsi qu'avec une approche plus nouvelle fondée sur du matériel d'analyse d'images, ont permis de montrer que le flétan se reproduit en groupe et au même moment et que le frai est le plus important en novembre–décembre, soit beaucoup plus tôt qu'on ne l'avait crû. Nos analyses nous permettent aussi de formuler quelques observations relatives au cycle biologique du flétan de l'Atlantique en eaux canadiennes. L'absence relative de géniteurs dans les secteurs du talus et du plateau continentaux pourrait signifier que le flétan se reproduit en eaux assez profondes, ce qui correspondrait assez bien aux moeurs du congénère du Pacifique, *H. stenolepis*. On trouve ordinairement des poissons immatures dans la partie sud-ouest du plateau Scotian, mais rarement au large de Terre-Neuve, ce qui confirmerait l'hypothèse selon laquelle le sud-ouest du plateau Scotian est une importante zone d'élevage du flétan immature.

Received February 20, 1992
Accepted September 14, 1992
(JB415)

Reçu le 20 février 1992
Accepté le 14 septembre 1992

Atlantic halibut (*Hippoglossus hippoglossus*) occurring off Canada's east coast are a significant resource, with annual landings valued at $15,000,000 in 1986 (Neilson et al. 1987). In spite of the economic importance of the stocks, comparatively little is known about their biology relative to that of other roundfish stocks. This is particularly true of their reproduction. While spawning is thought to take place in February, March, and April (McCracken 1958; Kohler 1967), the data were somewhat limited, as sample sizes were small and maturity was assessed using visual techniques only. While comparison of visual techniques with serological techniques for determination of maturity of the Pacific congener *H. stenolepis* have yielded consistent results, it has been observed that the use of small sample sizes for such studies may prove misleading, as they may represent early- or late-spawning components of the total population (G. St-Pierre, International North Pacific Halibut Commission, Seattle, WA, pers. comm.).

The location of spawning areas also remains poorly understood (Scott and Scott 1988). The absence of such information is of concern because management measures aimed at the conservation of Atlantic halibut stocks often have, as a prerequisite, knowledge of both the timing and location of spawning (Haug 1990).

This study, conducted as a joint effort between a fishermen's union and the Department of Fisheries and Oceans, the government agency responsible for research and management of

[1]Present address: CARICOM Fisheries Resource Assessment and Management Program, Pelagic and Reef Fish Resource Assessment Unit, Tyrell Street, Kingstown, St. Vincent, West Indies.

[2]Address for reprint requests.

[3]Present address: R.R. 2, Lower Hainesville, N.B. E0H 1J0, Canada.

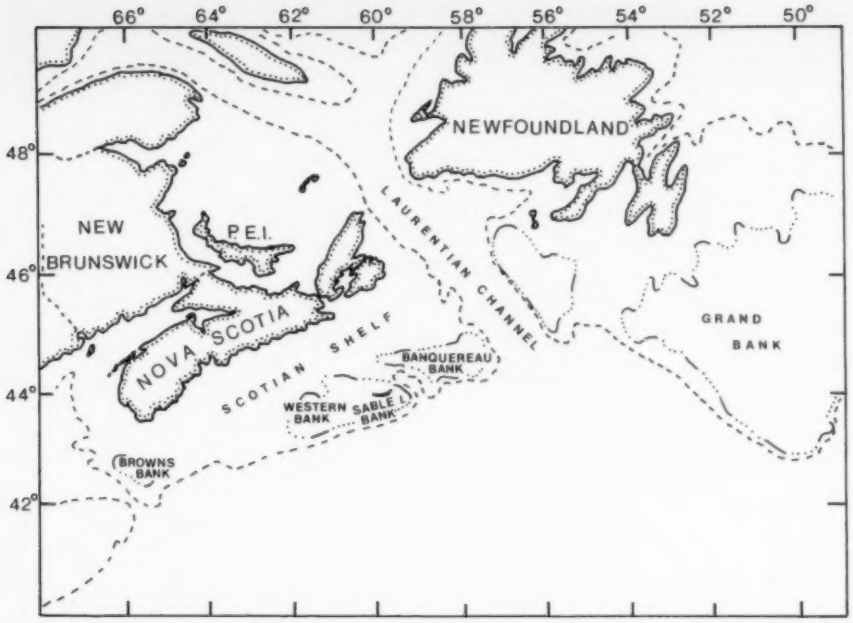

FIG. 1. Canadian North Atlantic, showing place names referred to in the text. The 100- and 200-m isobaths are also indicated.

the halibut stocks, was intended to address the fundamental deficiency in current understanding of Atlantic halibut reproduction. Our data also allowed us to elucidate aspects of Atlantic halibut life history which were previously uncertain. In particular, we documented the occurrence and distribution of Atlantic halibut of various maturity stages in the Scotian Shelf and Grand Bank areas. As a secondary objective, we also assessed the utility of image analyses systems (IAS) relative to more conventional techniques for studying fish reproduction.

Methods

Domestic Fishery Samples

Collections of halibut gonads were made from August 1988 to October 1989 by the crews of 52 fishing vessels throughout the Canadian northwest Atlantic. The study area and place names referred to here are shown in Fig. 1. While the majority of fishing vessels were longliners using hook and line gear, some material was also collected by otter trawlers. Gonads collected at sea were individually preserved in 10% neutrally buffered formalin provided in sampling kits given to participating fishermen. When practical, whole gonads were preserved. However, for later stages of maturation in particular, it was sometimes necessary to preserve a subsample (taken from the midpoint of the long axis) of the gonad only. Fishermen were also asked to complete labels providing information on gear type, date, fish length, and position (typically in the form of LORAN data). We converted LORAN data to map coordinates for plotting using a computer algorithm developed for the purpose.

Maturity stages of halibut were assigned by visual examination in a fashion identical to that described by Neilson et al. (1988) for other flatfish species, with eight maturity stages including, in chronological sequence, immature, ripening 1 (RI), ripening 2 (RII), ripe, spawning, spent, recovering, and resting. However, considerable uncertainty exists in the ability to separate immature and resting fish with such an approach.

An alternative approach using image analyses techniques for assessing the maturation stages of halibut ovaries was also attempted. A block of tissue approximately 8 cm^3 which included the ovary wall was excised from the midpoint of each ovary. Using a scalpel, oocytes were scraped off the block into a Petri dish with distilled water. Loose connective tissue, which tended to float, was decanted from the sample at this point. Initially, the oocytes that remained in the Petri dish were viewed with a stereomicroscope using transmitted light. A magnified (10–40×) and calibrated video image of 80–150 oocytes from each ovary was provided to a computer, where an algorithm traced the outline of each oocyte and calculated its area. Concurrent with their collection, oocyte area data were automatically recorded in a spreadsheet-type program for further analyses. Because the tracing of the egg perimeters occurred onscreen, the operator could detect and correct any errors immediately. We assessed the precision of the technique by making repeated measurements on the same oocytes after they were reoriented in the field of view. We also determined if the location of the sample of oocytes within the ovary affected the results.

To better describe the seasonal maturation of gonads and the mode of halibut reproduction, a subsample of 100 ovaries was examined using standard histological practices. Blocks of tissue were excised from the ovaries, serially dehydrated, and embedded in paraffin. Sections were cut at 4–6 μm and then stained with Harris' haematoxylin and counterstained with eosin.

Collections from Research Vessel Surveys

Data on the occurrence and distribution of various maturity stages of Atlantic halibut were obtained from regularly scheduled groundfish cruises occurring each March (1979–84), July

TABLE 1. Effect of varying sample location within Atlantic halibut ovaries for determination of average size of oocytes and its variation.

Maturity from visual examination	Location of sample within ovary	Mean oocyte area (mm^2)	Standard deviation
RI	Anterior	0.49	0.18
	Middle	0.52	0.20
	Posterior	0.43	0.16
RI	Anterior	0.13	0.18
	Middle	0.14	0.17
	Posterior	0.12	0.14
RI	Anterior	0.55	0.25
	Middle	0.46	0.22
	Posterior	0.33	0.19
RII	Anterior	2.16	0.29
	Middle	2.09	0.31
	Posterior	2.30	0.33
RII	Anterior	1.34	0.23
	Middle	1.32	0.23
	Posterior	1.33	0.24
RII	Anterior	2.40	0.22
	Middle	2.47	0.23
	Posterior	2.52	0.25

(1970–87), and October (1978–83). The March and October cruise series represent all data that are available. The July series, however, is ongoing. More recent data are not included here, as halibut catches have not been assessed with regard to maturity stage since 1987. The gear used on the surveys was the Western IIA bottom trawl, equipped with rollers. Sets were 30 min in duration, and the towing speed was 3.5 kn. Stations were selected according to a depth-stratified random sampling design (Halliday and Koeller 1981). Maturity stages were determined from visual inspection, as described earlier.

Typically, fish caught in such surveys were considerably smaller than those collected by fishermen using longline gear. Hence, the two datasets complement each other.

Collections from the International Observer Program

The third source of data for this study were observations of the length of halibut caught by offshore trawlers, collected through the International Observer Program (IOP). Such vessels typically operate at or outside the continental shelf break. The sex of halibut collected during IOP observations was not determined, nor was the maturity stage. The data available to us covered the period 1980–89.

Results

Domestic Fishery Samples

The data obtained using the IAS were, for a given ovary, unimodal and normally distributed. Thus, mean oocyte area was an appropriate statistic to describe central tendency. To assess the precision of measurements of oocyte area obtained with the IAS, we repeated measurements of the same 30 oocytes after obtaining a new video image of them five times. The coefficient of variation of the mean oocyte area calculated from the sample was 1.34%. To address whether oocyte area data would vary if the samples of oocytes were collected from a different part of the ovary than the midpoint we selected, we collected samples of oocytes from the anterior end, midpoint, and posterior end of the ovaries for six fish of different maturity stages (Table 1). From these data, little variation in average oocyte area as a function of where the sample was collected in the ovary was noted. What variation was present was small relative to the among-sample variation. We did, however, note that among late RII stage individuals, vitellogenesis was more complete at the posterior end of the ovaries, with a greater proportion of transparent oocytes present at the anterior tips of the ovaries.

We also compared the more traditional means of visual assessment of maturity with data from the image analyses system. While mean oocyte area data showed reasonable agreement with the assessment of maturity obtained by visual inspection, there were wide ranges of average oocyte areas within each maturity stage, which overlapped with adjacent stages (Fig. 2).

Table 2 illustrates the composition of the samples from the commercial fishery by month and by gear type. Seasonal coverage by the longline fleet was reasonably complete with the exception of November and December of 1988 and August, September, and October of 1989. Poor weather conditions and low availability of fish to longline gear resulted in no samples being collected during November and December 1988. Samples from trawlers were more intermittent. However, no concerted effort was made to obtain samples from that gear sector because such material would have duplicated, to some extent, the material available from the research vessel surveys.

The distribution of ovary samples from the commercial fleet is shown in Fig. 3A. Most samples originated near the shelf edge break around the 200-m isobath, with a considerable number of samples taken off southwestern Nova Scotia near Browns Bank. While most of the material originated from the Scotian Shelf, some samples were also collected adjacent to the Grand Banks of Newfoundland. In general, females in the early stages of maturation continued to be found in the vicinity of Browns Bank (Fig. 3B–3D) whereas ripe (Fig. 3E) and spent (Fig. 3F) individuals were rarely found inshore of the shelf edge. However, comparatively few fish in those advanced stages of maturity were caught.

Among the males sampled, we could determine the RI and RII maturation stages only from visual inspection. The distribution of samples of male halibut is shown in Fig. 4A and is broadly similar to the pattern of ovary samples collected (Fig. 3A). The distribution of RI males seemed to be in proportion to the distribution of the sampling effort (Fig. 4B). Very few RII samples were collected, but appeared to be concentrated near Browns Bank (Fig. 4C).

Information on the timing of reproduction was available from oocyte area data obtained using the IAS. These data are summarized in Fig. 5, stratified for several size-classes of females. The peak in oocyte size occurred either in or after October and was followed by a rapid decline in oocyte size to a minimum in the January–February–March period. Average oocyte area then increased through late summer. Thus, we interpret the peak spawning time to be, on average, the November–December period. The high variability associated with the January estimates (131–150 and 151–170 cm spawners) likely represents the incomplete release of oocytes among the population of spawners. We also found a relationship between mean oocyte area and the coefficient of variation associated with the mean. As oocyte area increased, the coefficient of variation decreased.

To examine whether there was a difference in the timing of spawning between the Scotian Shelf and the Grand Banks, we

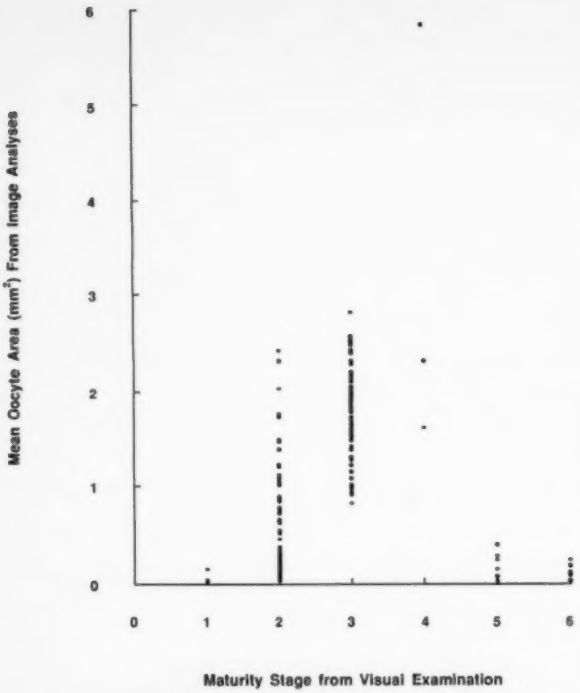

FIG. 2. Relationship between maturity stages of female Atlantic halibut obtained through visual inspection compared with the mean oocyte area obtained using image analyses techniques. Samples were collected from the commercial fishery operating in the Scotian Shelf and Grand Banks area, 1988–89. The maturity stages are coded on the *x*-axis label as follows: 1 = immature stage of development, 2 = ripening I (RI), 3 = ripening II (RII), 4 = ripe, 5 = spent, 6 = recovering.

selected only samples from the latter region. When mean oocyte area data were plotted, however, no difference in the timing of reproduction relative to the Scotian Shelf was apparent.

Examples of histological sections from immature, RI, RII, ripe, and recovering fish are shown in Fig. 6. The immature ovary contains a relatively homogeneous collection of oocytes at the beginning of their growth phases. The RI contains oocytes with multiple nucleoli within the enlarged nucleus (see smaller of two marked oocytes in Fig. 6). In RII, oocytes have nearly completed their yolk sequestering and have a solid yolk mass. The ripe phase is shown next. However, the example shown may contain earlier stages of oocyte development because the section provided is near the ovary wall. It is known that the oocytes typical of a more advanced condition are usually found near the lumen at the core of the ovary. The recovering ovary again consists of smaller oocytes, similar to the immature phase. The diagnostic feature that allows separation of the two stages, however, is the greater thickness of the ovary wall found in the recovering fish. On the basis of the appearance of different size-classes of oocytes in the histological sections, it appears that Atlantic halibut can be categorized as group-synchronous spawners (terminology of Wallace and Selman 1981).

Research Vessel Surveys

The spring, summer, and fall distributions of Atlantic halibut collected during groundfish surveys are shown in Fig. 7–9, respectively. Despite extensive coverage throughout the Scotian Shelf area, there were persistent concentrations of immature females (Fig. 7B, 8B, 9B) and males (Fig. 7D, 8D, 9D) both in the vicinity of Browns Bank and along the continental shelf break. Female halibut caught during RV surveys were either immature or recovering whereas the collections of males also included the RI stage of maturation.

International Observer Program

The distribution of Atlantic halibut noted during the IOP is shown in Fig. 10. Fish of the size-class <50 cm were virtually absent from the Grand Banks area except for three occurrences. Larger size-classes were generally distributed around the continental shelf margins of both the Scotian Shelf and the Grand Banks.

Discussion

Utility of IAS for Studies of Fish Reproduction Relative to Other Approaches

Measurements of oocyte development stage are often obtained by simply measuring oocyte diameter (West 1990). However, such an approach is complicated by the fact that teleost oocytes are rarely perfect spheres, necessitating several measurements of diameter. The automated approach of calculating oocyte area described here and in other papers (Witthames and Greer Walker 1987) eliminates the difficulty with estimating oocyte size through diameter measurements.

The utility of image analyses techniques such as we used here

TABLE 2. Summary of Atlantic halibut gonads collected with different gear types by fishermen, disaggregated by month, year and sex.

	1988					1989										
Gear type	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Total
							Female									
Longline	33	146	56	0	0	57	35	11	30	6	23	14	0	0	1	412
Trawl	1	0	1	0	0	31	0	6	0	0	0	0	0	0	0	39
Total	34	146	57	0	0	88	35	17	30	6	23	14	0	0	1	451
							Male									
Longline	23	63	7	0	0	57	45	32	20	3	10	29	0	0	0	289
Trawl	0	0	0	0	0	16	0	2	0	0	0	0	0	0	0	18
Total	23	63	7	0	0	73	45	34	20	3	10	29	0	0	0	307
Grand total	57	209	64	0	0	161	80	51	50	9	33	43	0	0	1	758

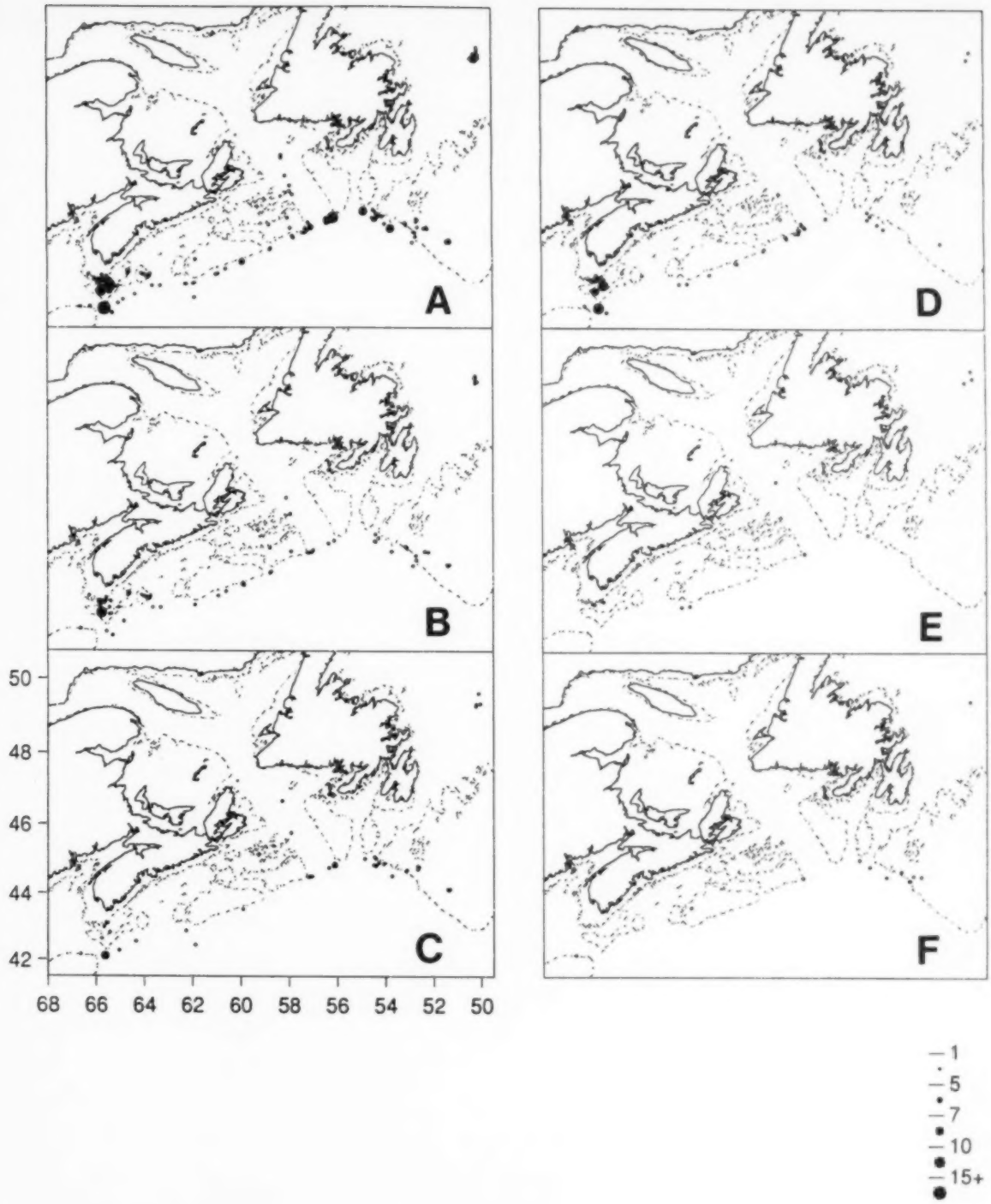

FIG. 3. (A) Samples of female halibut obtained by commercial fishermen in 1988–89, (B) immature stage of development, (C) RI, (D) RII, (E) ripe, and (F) recovering. Samples were collected from the Scotian Shelf and Grand Banks areas. The 100-m isobath is also shown.

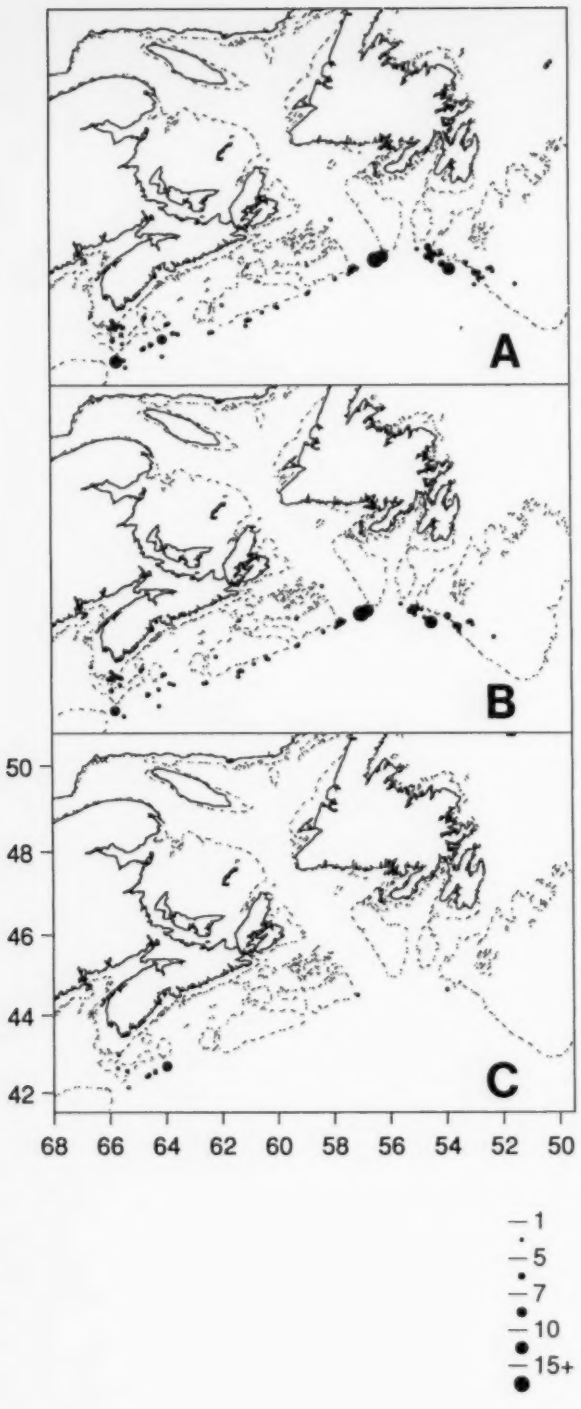

FIG. 4. (A) Samples of male halibut obtained by commercial fishermen in 1988–89, (B) RI, and (C) RII. Samples were collected from the Scotian Shelf and Grand Banks areas. The 100-m isobath is also shown.

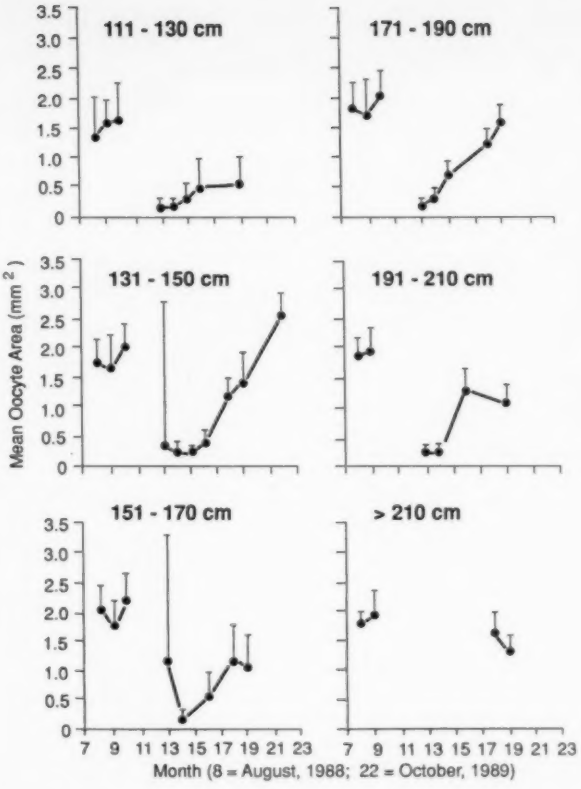

FIG. 5. Mean oocyte areas (± 1 SD) versus month of capture, August 1988 to October 1989. Each mean consists of 75–150 individual oocyte measurements, and the number of samples comprising each mean is shown in Table 2. Samples were collected from the commercial fishery operating in the Scotian Shelf and Grand Banks areas.

for oocyte area measurements compared favourably with other automated systems based on light transmission. For example, such systems often rely upon measurements of size based on the area of the shadow cast as an oocyte moves through the region of the sensor (Davis 1984; Witthames and Greer Walker 1987). If such oocytes are not sufficiently opaque, as might be the case with those with incomplete vitellogenesis, oocyte sizes will be underestimated. With image analyses techniques such as ours, the operator can easily compare the actual oocyte dimensions with those obtained using the IAS during the data-gathering process, thus avoiding the problem described above.

IAS-based approaches also compare favourably with histology for certain investigations of fish reproduction. For example, a well-known problem with estimates of oocyte size obtained using histology is whether oocytes are sectioned through the plane of maximum area (Foucher and Beamish 1980). As our approach involved the examination of whole oocytes, such a problem is not a concern. The IAS-based method also has the advantages of speed of processing the samples and does not necessitate the use of noxious materials such as xylene, a common requirement of histological processing. On the other hand, histology can obviously provide more information on internal microstructural detail than is possible using IAS on whole oocytes.

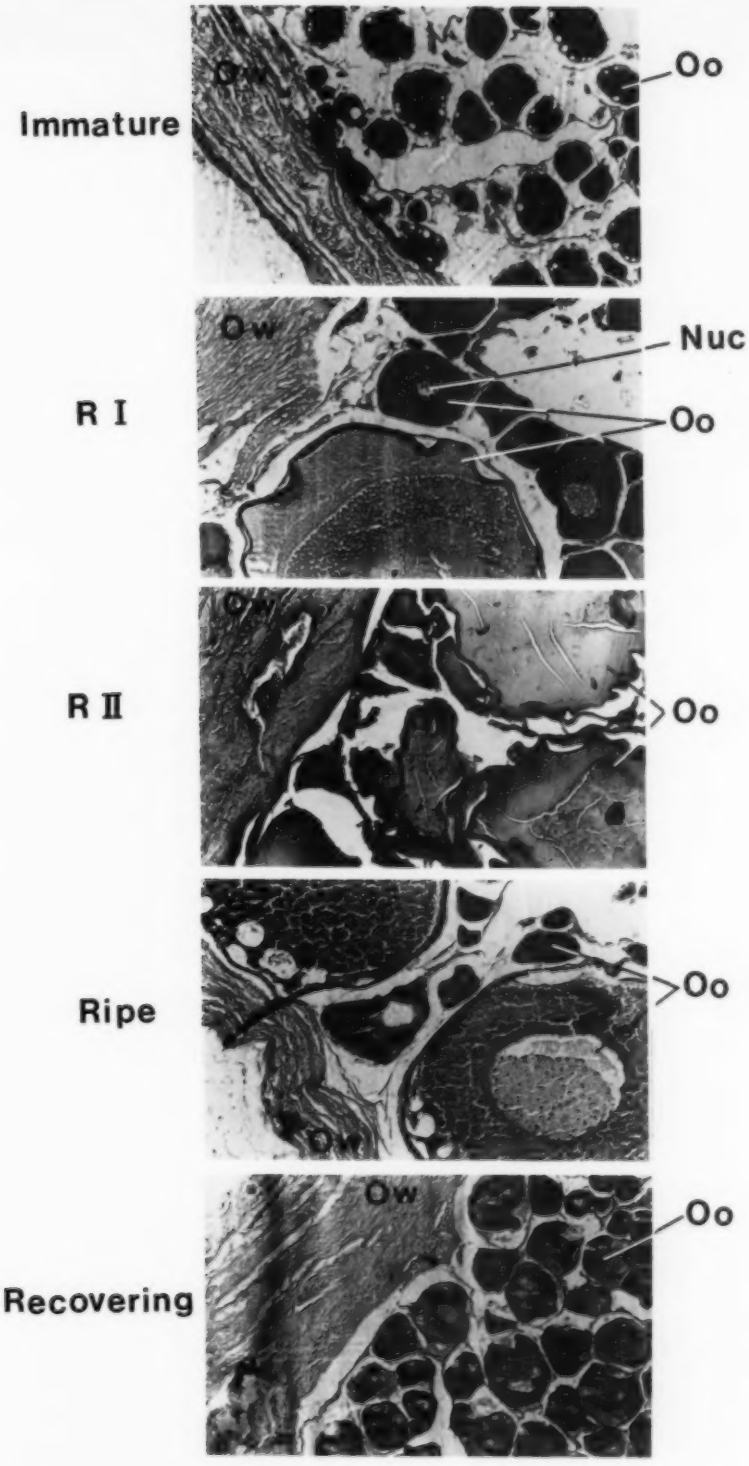

FIG. 6. Histological sections of Atlantic halibut ovaries of varying maturity stages, ranging from immature through recovering. Oo, oocytes; Ow, ovarian wall; Nuc, multiple nucleoli. Samples were collected from the commercial fishery operating in the Scotian Shelf and Grand Banks areas, 1988–89.

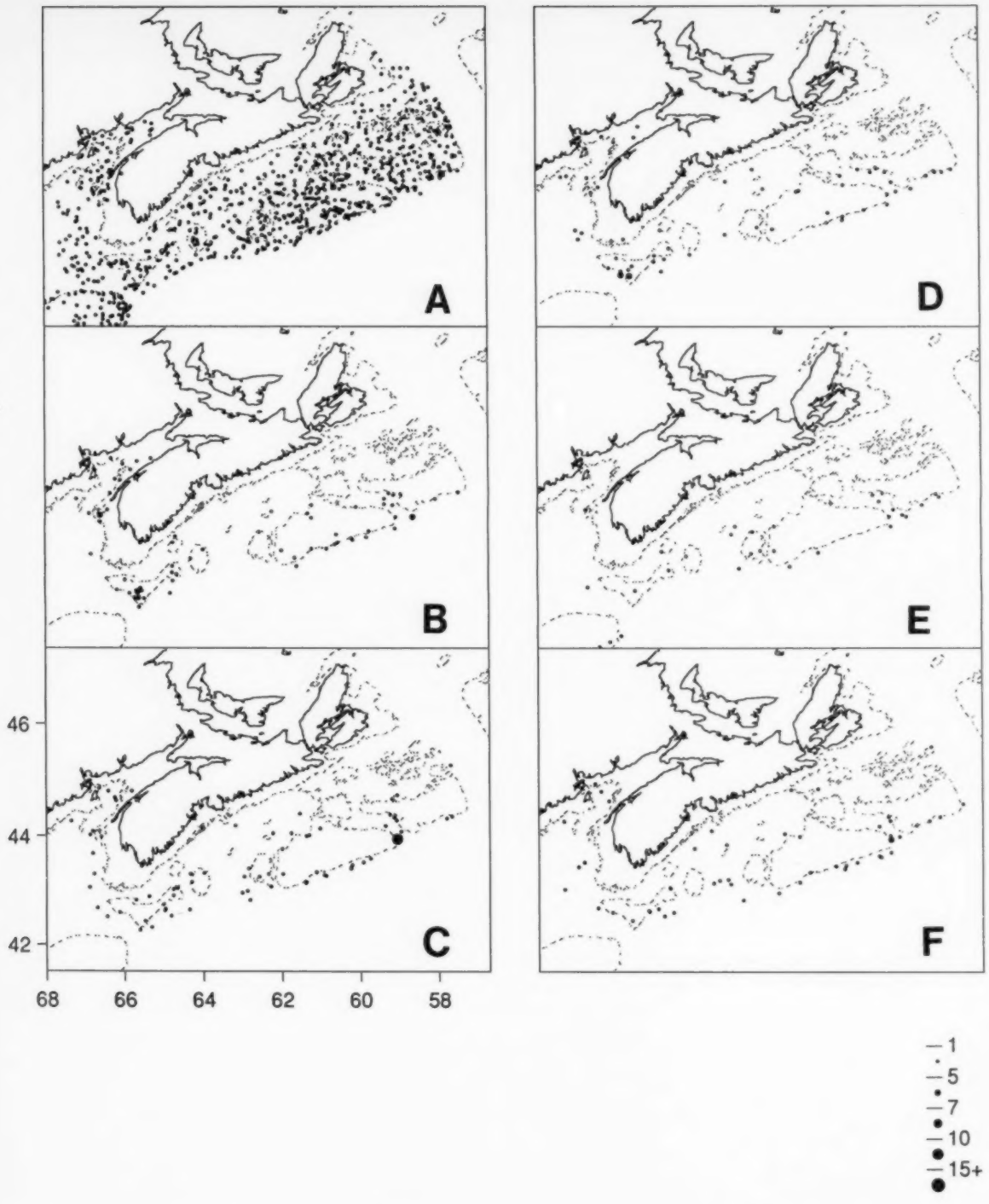

FIG. 7. (A) Distribution of sets during research vessel surveys of groundfish abundance on the Scotian Shelf conducted in March 1979–84; (B) distribution of immature female Atlantic halibut caught during such surveys; (C) female recovering stage; (D) distribution of immature male Atlantic halibut; (E) RI; (F) recovering stage. The 100-m isobath is also shown.

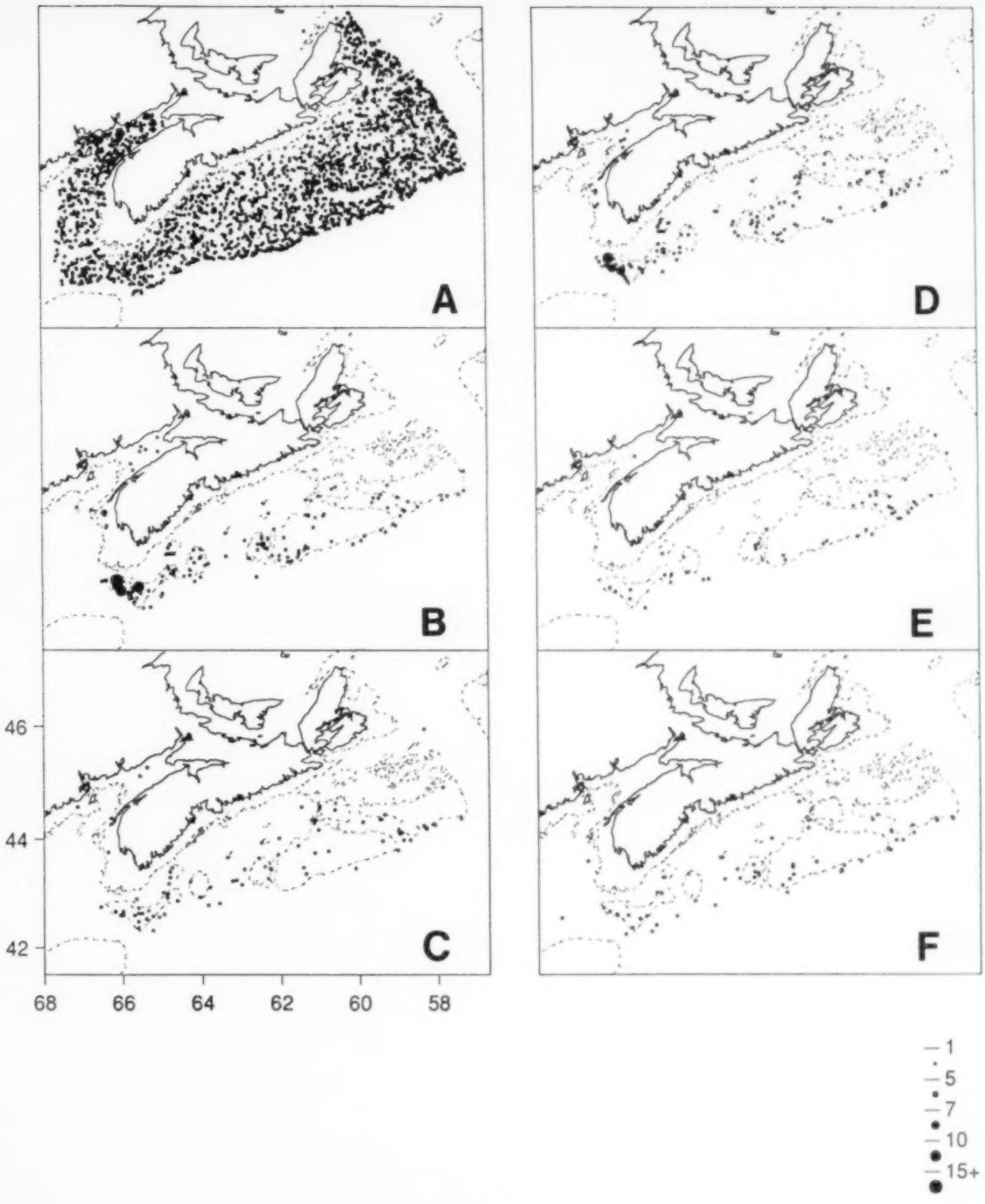

FIG. 8. (A) Distribution of sets during research vessel surveys of groundfish abundance on the Scotian Shelf conducted in July 1970–87; (B) distribution of immature female Atlantic halibut caught during such surveys; (C) female recovering stage; (D) distribution of immature male Atlantic halibut; (E) RI; (F) recovering stage. The 100-m isobath is also shown.

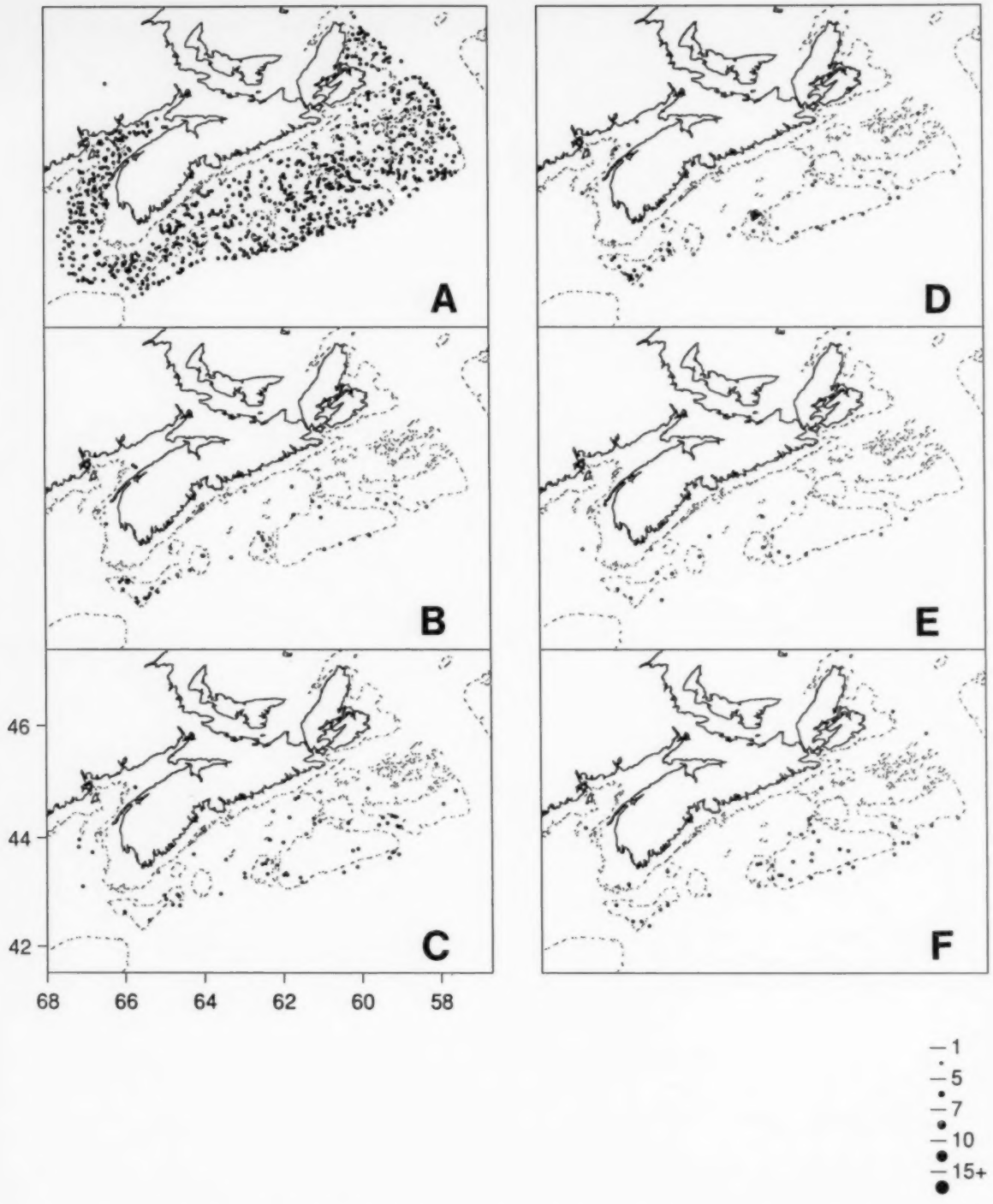

FIG. 9. (A) Distribution of sets during research vessel surveys of groundfish abundance on the Scotian Shelf conducted in October 1978–83; (B) distribution of immature female Atlantic halibut caught during such surveys; (C) female recovering stage; (D) distribution of immature male Atlantic halibut; (E) RI; (F) recovering stage. The 100-m isobath is also shown.

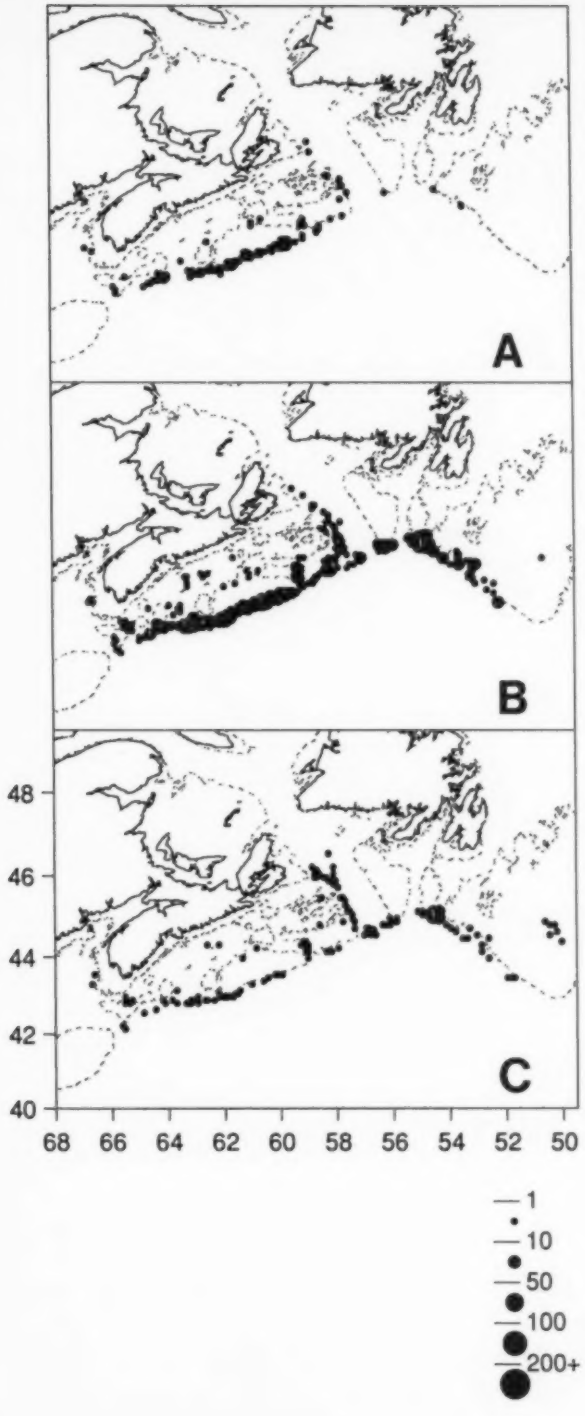

FIG. 10. Catch distribution of Atlantic halibut (A) less than 50 cm, (B) 50–100 cm, and (C) 101–150 cm noted by observers stationed onboard fishing vessels operating off the Scotian Shelf and Newfoundland, 1980–89. The 100-m isobath is also shown.

There was some agreement between data obtained using the IAS and visual inspection of the ovaries (Fig. 2), at least for the RI and RII maturation stages, although considerable overlap was found. We interpret this as indicating that assessments of maturity based on visual inspection may, on the average, be an acceptable means of assessing the onset of spawning. However, as is well known (West 1990), such techniques do not allow the discrimination of immature and spent ovaries with acceptable accuracy.

We also found a negative correlation between mean oocyte area and the coefficient of variation associated with the mean. This could be interpreted as a pattern of resorption or atresia as gonad maturation continues. Just prior to release of oocytes into the lumen, the ovary consists of uniformly sized oocytes, with comparatively few smaller oocytes with incomplete yolk sequestering. However, given the histological evidence presented in Fig. 6 wherein several size-classes of oocytes are well represented, even in the ripe ovary, an alternative explanation is required. We suggest that the data obtained using the IAS-based approach represent, in general, vitellogenic oocytes. In preparing the samples as described in the Methods section, we therefore selectively removed such oocytes, omitting the smaller size-classes. Our conclusions regarding halibut reproductive biology are unaffected by such a potential bias, however, as we were interested in obtaining the largest oocytes to assess the timing of spawning. In fact, most authors who have used oocyte size as an indicator of development have selected data from the largest mode of observations for group-synchronous spawners (West 1990).

Atlantic Halibut Reproductive Biology

Atlantic halibut occurring in the northwest Atlantic are annual, group-synchronous spawners. This conclusion is consistent with the observation of Haug and Gulliksen (1988) for halibut occurring in the northeast Atlantic, who found that spawning is intermittent, with only a proportion of the maturing oocytes undergoing hydration and being discharged at any one time. Atlantic halibut held in captivity produce batches of eggs which vary in number and are released at more or less regular intervals throughout the spawning season (Haug 1990). The Pacific congener *H. stenolepis* is also known to be an annual spawner demonstrating the group-synchronous mode of reproduction (St-Pierre 1984).

On average, peak spawning activity occurs in the November–December period. Our results do not support earlier studies of the timing of spawning of Atlantic halibut. McCracken (1958) found peak spawning around March or April on the Scotian Shelf, but this conclusion appears to have been reached from examination of seven gonads only, all collected during the April–May period. McCracken also examined 29 ovaries during the September and October period and found them to be relatively turgid, which we suggest is consistent with imminent spawning. Kohler (1967) agreed that spawning on the Scotian Shelf took place in late winter and early spring, although his sample size was also small (a total of 53 females from collections made during 3 mo). More consistent with our findings, St-Pierre (1984) found spawning in Pacific halibut to take place between November–March, with the period of greatest spawning activity occurring between the last week of December and the third week of January. Further indirect support of our conclusion that halibut spawning occurs in November–December is the lack of availability of fish to the gear during that period. It is known that halibut feeding activity is much reduced during

spawning (Haug 1990); hence, their vulnerability to capture with longline gear would be correspondingly decreased.

Earlier papers than those described above, however, provide narrative support of our view of the timing of reproduction of Atlantic halibut. Goode and Collins (1887) noted that on the Grand Bank of Newfoundland, halibut have been found just previous to and during their spawning season, in July, August, and September, along the slopes of the outer banks. Goode also presented evidence of a spring and fall migration between relatively shallow feeding grounds and deeper spawning grounds. Hjort (1905) stated that halibut spawn during November in Norwegian waters at depths between 250 and 300 fathoms. Devold (1938) described how Norwegian fishermen caught halibut in spawning condition around Christmas. Rae (1959) gave two views of the timing of halibut, first noting that halibut in the northeast Atlantic spawn around April and May, but also reporting that three catches were obtained off western Iceland in December which consisted almost exclusively of large ripe female halibut, with fully developed ovaries.

Our samples of halibut contained proportionately few individuals in a ripe state of maturity. We suggest that in the northwest Atlantic, halibut spawn in deep water off the continental shelf, beyond the area covered in our study. Such an interpretation is consistent with data from the northeast Atlantic. For example, Haug (1990) reported that halibut off the Faroe Bank in the northeast Atlantic spawn at a depth of 700–1000 m. Further support for the view that spawning occurs offshore in deep water comes from the Scotian Shelf Ichthyoplankton Program (SSIP), an extensive series of ichthyoplankton surveys conducted throughout each season from 1978 to 1982 (Neilson et al. 1988). Typically, relatively few stations were occupied beyond the 200-m contour near the edge of the Scotian Shelf. A similar broad-scale program known as the Fisheries Ecology Program focussed on the vicinity of Browns Bank and continued from 1983 to 1985 (Dickie and Smith 1989). In the course of all surveys, no halibut eggs and only three and two larvae were caught for the SSIP and FEP cruises, respectively (P. Hurley, Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.).

While the shallow-water areas of the Canadian continental shelf do not appear to be spawning areas for Atlantic halibut, ularly the area near Browns Bank, may serve as such a nursery area for immature halibut in the Canadian northwest Atlantic. To further support our assertion, we show the mean size of Atlantic halibut caught during seasonal research vessel surveys, by North Atlantic Fisheries Organization (NAFO) unit area (Fig. 11). The smallest average length of halibut was usually taken in the unit area 4Xp, corresponding to Browns Bank and its vicinity. The postulated nursery ground in the vicinity of Browns Bank is consistent with reports of catches of uniformly small (30–50 cm), immature halibut from this area (G. Donaldson, Marine Fish Division, Yarmouth, N.S., pers. comm.).

The comparative absence of small halibut in Newfoundland waters (Fig. 10A) is also of significance. We suggest that many of the larger fish found on the Grand Bank may have originated from the Scotian Shelf nursery area. This view of halibut life history, although speculative, is supported by available tagging data. Stobo et al. (1988) demonstrated a tendency for Atlantic halibut tagged on the Scotian Shelf to move northeast of the release site, with a significant number of recaptures occurring on or near the Grand Banks. Those authors also indicated that it was, on average, smaller fish that appeared to be the most

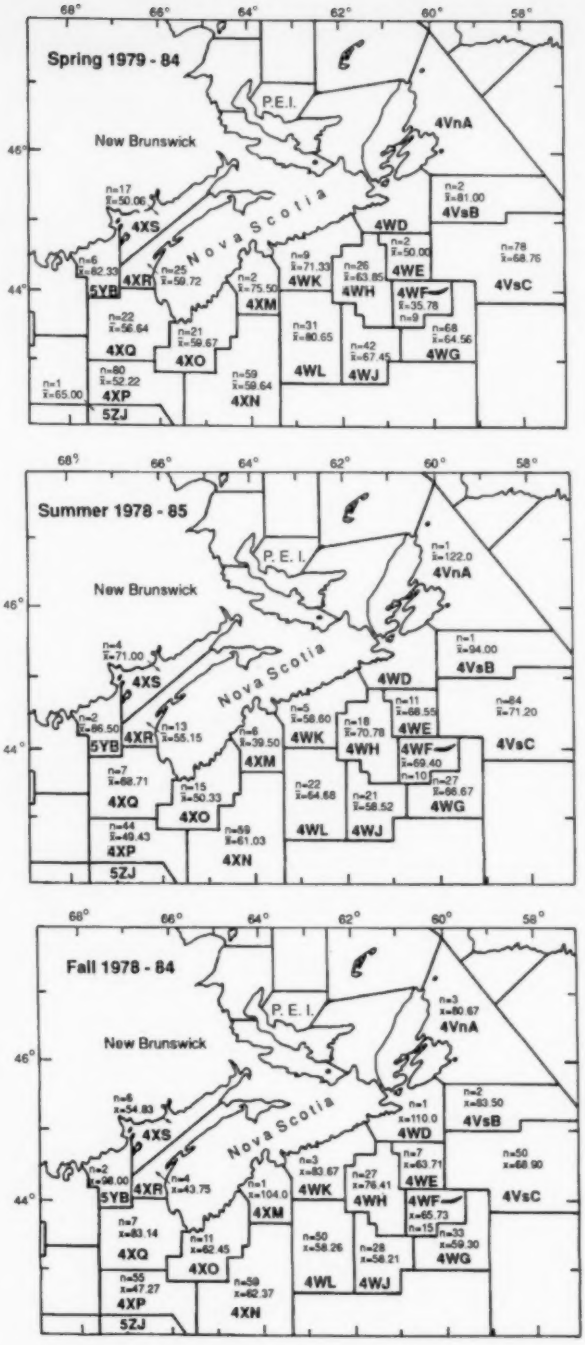

FIG. 11. Average size and number caught of Atlantic halibut caught during research vessel surveys of groundfish abundance on the Scotian Shelf conducted in spring, 1979–84, summer 1979–85, and fall 1978–84, shown by North Atlantic Fisheries Organization (NAFO) unit area.

vagrant. Such evidence, taken together with other published work and the present study, gives rise to the following life history scenario: Atlantic halibut spawn in relatively deep water off the continental shelf, rear in shallow inshore or shelf areas

for 3–4 yr (Godø and Haug 1988), and then enter a highly migratory phase that appears to coincide with a shift in diet preference to almost exclusive piscivory (Trumble et al. 1993); when sexual maturity is reached at an age of 8–12 yr (Bowering 1986), halibut move to spawning grounds, and there is indication of a homing response among repeat spawners (Haug 1990).

The interpretation of Atlantic halibut life history presented here has important implications for the management of the fishery. For example, the small, immature fish caught in the Browns Bank area are often taken as bycatch for other groundfish fisheries (G. Donaldson, Marine Fish Division, Yarmouth, N.S., pers. comm.). The recent introduction of an 81-cm minimum size limit, originally justified on the basis of increased value of landings to fishermen (Neilson and Bowering 1989), should reduce wastage of such immature fish. A second benefit of the minimum size regulation would be a potential increase in the numbers of spawners, if the released immature fish survive to sexual maturity. A further management implication of our findings is that if the postulated deep-water spawning areas for Atlantic halibut exist, it would mean that spawning aggregations such as those described by Haug (1990) are not subject to the comparatively intense exploitation of the shallower-water bank fisheries. This feature of halibut life history may help sustain populations in the northwest Atlantic.

Acknowledgments

We thank the many vessel owners and fishing companies who assisted with this study. Without their cooperation, this work would not have been possible. We also thank Mark Showell of the International Observer Program for extracting data of interest to us. Dany Lise Langlois, Peter Comeau, and Allyson Cushing coordinated field collections. Drs. E. Trippel and F. Page provided useful critical comments on an earlier version of this manuscript. In particular, we wish to thank Dr. Gilbert St-Pierre of the International Pacific Halibut Commission for bringing several key references to our attention and for his careful review of the manuscript.

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Spatial and Parent-Age Analysis of Stock–Recruitment in the Georges Bank Sea Scallop (*Placopecten magellanicus*) Population

Richard McGarvey[1]

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada

Fredric M. Serchuk

National Marine Fisheries Service, Northeast Fisheries Center, Woods Hole, MA 02543, USA

and Ian A. McLaren

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada

McGarvey, R., F. M. Serchuk, and I. A. McLaren. 1993. Spatial and parent-age analysis of stock–recruitment in the Georges Bank sea scallop (*Placopecten magellanicus*) population. Can. J. Fish. Aquat. Sci. 50: 564–574.

Reproductive interaction between and within subpopulations on Georges Bank was investigated employing annual sampling surveys of sea scallop (*Placopecten magellanicus*) abundance available from 1977 to 1988. Time series of recruitment and, together with size-specific fecundity, total yearly egg production were constructed for Georges Bank and all subregions to generate a spatial stock–recruitment analysis. Recruitment is correlated with egg production within the population on the Northern Edge and Northeast Peak which may imply that it is reproductively self-sustaining. Stock–recruitment correlations increased when the eggs of scallops age 3 and 4 were excluded from the stock measure of total egg production. This increase in correlation occurred in every subregion where there was evidence of a positive relationship between eggs and recruits, most strongly for the Northern Edge and Northeast Peak. This is consistent with the hypothesis that older scallops (ages 5+) are the principle contributors to recruitment. Presently, the fishery begins removing large numbers at age 3.25. Previous yield per recruit analyses have predicted higher yields if age of first capture were raised. These correlations may suggest that the same policy could also result in higher recruitment.

À partir des dénombrements annuels par échantillonnage des pétoncles (*Placopecten magellanicus*) pour la période de 1977–1988 dont nous disposions, nous avons étudié, à l'intérieur de sous-populations et entre des sous-populations du banc Georges, les rapports qui ont trait à la reproduction. Nous avons constitué des séries chronologiques du recrutement et, avec la fécondité par groupe de taille, la production annuelle totale d'oeufs dans le secteur du banc Georges et dans toutes les sous-régions desquelles on a pu tirer une analyse spatiale complète des stocks et du recrutement. Le recrutement est mis en corrélation avec la production d'oeufs au sein de la population de la bordure nord et de la pointe nord-est; cela pourrait signifier que cette population parvient à se maintenir seule. Les corrélations entre le recrutement et les stocks se trouvaient renforcées lorsque les oeufs des pétoncles de 3 et 4 ans étaient exclus de la mesure de la production totale d'oeufs dans chacune des sous-régions où il paraissait y avoir un rapport positif entre les oeufs et les recrues, particulièrement dans le secteur de la bordure nord et de la pointe nord-est. Cela vient conforter l'hypothèse selon laquelle les pétoncles les plus âgés (5 ans et plus) sont ceux qui contribuent le plus au recrutement. À l'heure actuelle, les pêcheurs font des prélèvements importants à partir de l'âge de 3,25 ans. Les précédentes analyses de rendement par recrue laissaient prévoir qu'un relèvement de l'âge de la première capture conduirait à des rendements supérieurs. Ces corrélations pourraient indiquer que la même politique pourrait aussi conduire à un recrutement supérieur.

Received June 24, 1991
Accepted September 17, 1992
(JB098)

Reçu le 24 juin 1991
Accepté le 17 septembre 1992

The sea scallop (*Placopecten magellanicus*) populations on Georges Bank (Fig. 1) are the largest, densest, and most intensively exploited of this species. They are consistently found in three specific regions: the Northern Edge and Northeast Peak, the Southeast Part, and the Great South Channel (Sinclair et al. 1985). Understanding spatial reproductive interaction, including the principal sources of spawn for each aggregation, is important in managing this valuable (~$100 million per year) resource. We inquire how recruitment year classes depend on egg production within each region and on neighboring subpopulations. The time averages of recruitment, egg production, biomass, and population numbers in each region, as densities and absolute abundances, were computed in a previous work (McGarvey et al. 1992).

Scallops spawn yearly, in early fall (Naidu 1970; Robinson et al. 1981; Beninger 1987), older females releasing up to 200 million eggs (Langton et al. 1987). Fecundity increases allometrically with age (MacDonald and Thompson 1985a, 1985b; Langton et al. 1987) and varies from year to year about twofold due to changing conditions in the water column, particularly phytoplankton food supply and water temperature (MacDonald and Thompson 1986a, 1986b, 1988; MacDonald et al. 1987; Shumway et al. 1987; Barber et al. 1988). Planktonic larvae,

[1]Present address: Chesapeake Biological Laboratory, P.O. Box 38, Solomons, MD 20688, USA.

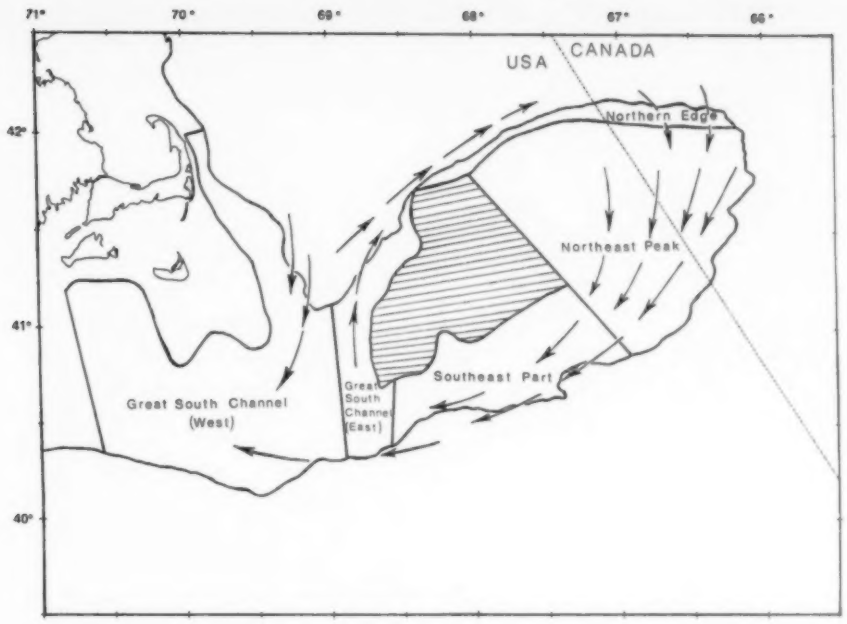

FIG. 1. Georges Bank partitioned into five subregions along the clockwise flow of residual current.

grazing on phytoplankton, settle on the bottom after 3–6 wk (Culliney 1974). The survival rate from egg to age 2 juvenile is very low, presently about 1 in 10 million (McGarvey et al. 1992). Predation, starvation in the water column, temperature, and settlement on uninhabitable bottom all probably contribute to high early life history mortality.

Sinclair et al. (1985) reasoned that throughout the Northwest Atlantic, and in particular in each of the three regions on Georges Bank, scallop populations must be self-sustaining because they have persisted at those locations over long times. Surveying larval abundance in the Bay of Fundy, Tremblay and Sinclair (1988) concluded that larvae were predominantly spawned by and subsequently settle in long-exploited aggregations where tidally driven semiclosed currents at the mouth of the Bay of Fundy would be expected to transport them. On the Northern Edge and Northeast Peak of Georges Bank, where the population is considerably larger, Tremblay and Sinclair (1990) found larval concentrations an order of magnitude more dense than in the Bay of Fundy.

Zouros and Gartner-Kepkay (1985) found no significant allozyme differentiation, implying no evidence of genetic differentiation, among 10 *Placopecten* populations near Nova Scotia, including the three on Georges Bank. Subpopulations could still prove to be reproductively isolated if distinguishing genetic markers are found. However, the time required for a neutral mutation to become fixed is of the order of 4 times the mean age of reproduction times population size. If more than one individual per generation migrates between populations, differentiation by random genetic drift is effectively excluded (Hartl and Clark 1989). Mean age of reproduction is 5 yr. These subpopulations range in size from 65 million to over 1 billion (McGarvey et al. 1992). Furthermore, residual currents on Georges Bank probably carry an average larva distances comparable with those between these neighboring populations every spawning season (Posgay 1979). Observed population and migration patterns are thus in accord with the lack of genetic differentiation found by Zouros and Gartner-Kepkay (1985).

Butman and Beardsley (1987) and Butman et al. (1987) confirmed that the prevailing residual current on Georges Bank, driven by the diurnal tidal cycle (Greenberg 1983), is a clockwise gyre (Fig. 1). Spatial models incorporating the semiclosed circulation of water above Georges Bank have been used to analyze copepod development (Davis 1984) and the scallop fishery (Caddy 1975).

Because of the clockwise movement of water on Georges Bank, an alternative hypothesis to reproductive isolation is that larvae are carried in substantial numbers between neighboring subpopulations. We investigate this possibility by comparing stock–recruitment correlations within each regional subpopulation with those between neighboring subregions, particularly bearing in mind the clockwise direction of the residual current. The subpopulation on the Northern Edge and Northeast Peak comprises 84% of the Georges Bank total (McGarvey et al. 1992) and is therefore the primary focus of our investigation. We address the three following questions: Is there a stock–recruitment relationship? What is its spatial pattern on Georges Bank? How is recuitment affected by the age/size of the parent stock? In doing so, we address a fourth question: Does recruitment variability exhibit a consistent distribution?

Recruitment Model

Three stages in early life history determine yearly recruitment to age 2: (1) total (fertilized) egg production, S, (2) density-independent survival in the water column, L_{wc}, and (3) density-dependent spatfall and survival to age 2, $L_{dd}(S)$. The equation for the number of 2-yr-old recruits spawned in year y is therefore

$$(1) \quad R2_{y+2} = S_y \cdot L_{wc_y} \cdot L_{dd}(S).$$

This paper investigates the evidence for the first factor, i.e. that recruit numbers are correlated with annual egg production.

Large year-to-year variation in survival during the pelagic larval stage, L_{wc_y}, is primarily driven by changes in the pattern of current flow. Its pelagic trajectory in the month following spawning determines if a scallop egg or larva is carried into regions of low predator and high phytoplankton density to optimum temperatures and to settlement on favorable bottom habitat. L_{wc} is itself the product of survivals in the stages of fertilization, free-floating egg, feeding larva, and settlement. Annual variation in the Labrador Current, warm-core rings (Halliwell and Mooers 1979; Myers and Drinkwater 1989), the 18.6-yr tidal cycle (Loder and Garrett 1978), and wind patterns (Greenberg 1983; Hopkins and Raman 1987), combined with the potentially chaotic instability of fluid dynamics, can all significantly alter the prevailing flow above Georges Bank. Assuming negligible interaction among larvae and between larvae and adults in the water column, these current-driven processes are density independent. The effect of this marine environmental variability on recruitment is, in many cases, stronger than egg production. This model postulates the multiplicative influence of both.

Because adult scallops have been heavily fished during the years analyzed here, 1977–88, density-dependent factors have played a smaller role than they would in an unexploited population. Assuming densities have been kept below the carrying capacity in most beds on Georges Bank, we neglect density dependence in this study and test only for an increasing relationship between S_y and $R2_{y+2}$.

Data

Since 1977, annual scallop surveys have been carried out on Georges Bank by Canada and the United States (Serchuk and Wigley 1989; Robert and Black 1990). At several hundred sampling locations, a commercial scallop drag, fitted with a smaller mesh liner, is towed along the bottom for a fixed distance. Yielding a sample of some 50,000, scallops are measured for shell height and the numbers in each size class of 0–5, 5–10 mm, etc., at each sampling location are recorded (Fig. 2).

Analyzing the precision of these height–frequency data, Serchuk and Wigley (1986; see also Mohn et al. 1987) compared coincident US and Canadian scallop surveys in the Northern Edge and Northeast Peak from 1982 to 1984. The 95% confidence intervals, derived from the deviation of these two independent survey means, yielded ±17, ±27, and ±5% for recruitment numbers in 1982, 1983, and 1984 (McGarvey et al. 1992) when the means themselves varied 600%. This accuracy was higher than predicted from the two sampling standard deviations which each yield an independent estimate of confidence interval (McGarvey et al. 1992). Commercial catch per unit effort, unbiased in this fishery by catch restrictions until recently, agrees ($r = 0.905$, $P = 0.00007$, df = 9) with these measures of survey abundance (McGarvey 1991). It would seem likely that the large sample sizes, and the relative immobility (Baird 1954, Posgay 1963; Melvin et al. 1985) and strictly two-dimensional distribution of scallops in contrast with finfish, contribute to the apparent high accuracy of these survey data.

Methods

The time series of parent stock (i.e. total egg production), $\{S_y\}$, and recruitment to age 2, $\{R2_y\}$, were derived from the

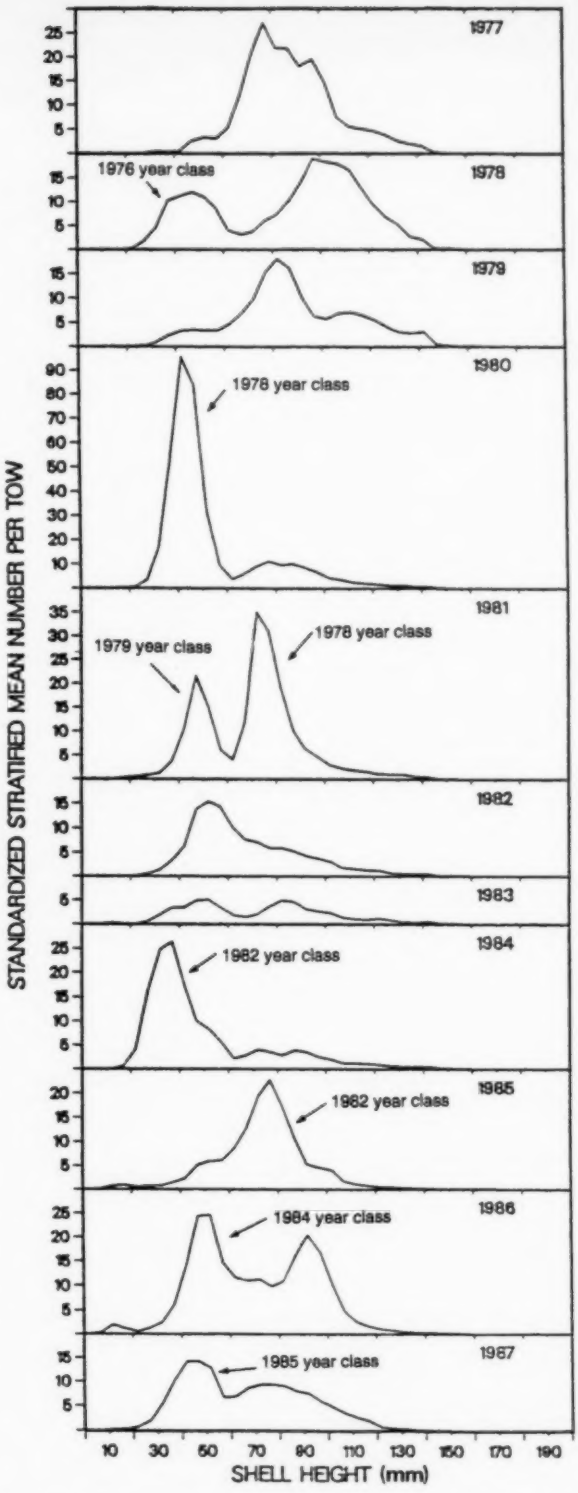

FIG. 2. Survey height–frequency distributions for Georges Bank scallops, 1977–87.

survey height–frequencies from 1977 to 1988 in each region as follows.

Annual egg production in year y was calculated as

$$S_y = \sum_{h = 40\text{ mm}}^{185\text{ mm}} N_{y,h} \cdot e_h.$$

$\{N_{y,h}; Y = 1977, \ldots, 1988; h = 0\text{–}5, 5\text{–}10, \ldots, 180\text{–}185\}$ is the matrix of survey data, the stratified mean number of scallops gathered per tow each year, y, for each scallop height class, h, in each region. Fecundity as a function of shell height, $\{e_h; h = 0\text{–}5, 5\text{–}10, \ldots, 180\text{–}185\}$, was derived (McGarvey et al. 1992) from field studies of scallop reproductive energetics (MacDonald and Thompson 1985b).

Summing numbers per tow under the first identifiable year-class peak in the height–frequency distributions (Fig. 2), namely over the shell height size range of 30–60 mm, yielded age 2 numbers per tow

$$R2_y = \sum_{h = 30\text{ mm}}^{60\text{ mm}} N_{y,h}$$

for each survey year from 1977 to 1988. Lagging back 2 yr, the identical recruitment time series, $\{R_y = R2_{y+2}; y = 1975, \ldots, 1986\}$, identifies recruits by the year, y, they were spawned. $\{R_y\}$ was then compared with $\{S_y\}$ within and between regions, with correlation and significance calculated for each case. Because we calculate only correlations, the results presented below would be unaffected if S and R were rescaled to absolute population numbers or densities.

Because there is also strong environmental variability affecting R, the absolute levels of correlation with S can be uncertain measures of the influence of egg production on recruitment. Moreover, relatively uniform measurement bias, particularly variation in sample capture efficiency with size, may affect the R time series (McGarvey et al. 1992). Therefore, *differences* in stock–recruitment correlations can be more meaningful and reliable than their absolute values. In this study, spatial partitions into one, three, and five subregions and a further redefinition of egg production into four different minimum ages of parent stock provide the opportunity to investigate potential trends in these stock–recruitment correlations. This approach was made possible by the large sample sizes of location- and size-specific survey data.

In assessing the extent of larval transport between subpopulations, absolute stock–recruitment correlations can yield only a positive answer about the reproductive relationship between subregions: the eggs from one area may be the principal source of recruits to a downstream population yet yield no positive correlation, since the effect of egg production may be obscured by a stronger environmental effect. On the other hand, the stronger the absolute stock–recruitment correlation, the more likely it is that larval transport is substantial.

Dependence of Recruitment Success on Age of Parent Stock

We constructed three additional measures of parent stock which count eggs produced by scallops of ages 4 and older, ages 5 and older, and ages 6 and older:

$$S4+_y = \sum_{h = 90\text{ mm}}^{185\text{ mm}} N_{y,h} \cdot e_h$$

$$S5+_y = \sum_{h = 105\text{ mm}}^{185\text{ mm}} N_{y,h} \cdot e_h$$

TABLE 1. Test of normality and lognormality of recruitment and stock frequency distributions from all subregions.

Region	Significance of Shapiro and Wilk's W-test for normality		
	R	S	$S5+$
	ln(R)	ln(S)	ln($S5+$)
Georges Bank	0.00046	0.20	0.0035
	0.44	0.76	0.11
Northern Edge and Northeast Peak	0.0014	0.14	0.00092
	0.50	0.78	0.22
Northern Edge	0.00004	0.008	0.00093
	0.23	0.37	0.03
Northeast Peak	0.0048	0.037	0.00085
	0.82	0.70	0.26
Southeast Part	0.23	0.28	0.015
	0.24	0.52	0.81
South Channel (both halves)	0.014	0.30	0.0051
	0.66	0.76	0.019
Eastern half of South Channel	0.073	0.0015	0.0019
	0.36	0.13	0.16
Western half of South Channel	0.009	0.22	0.098
	0.51	0.75	0.52

$$S6+_y = \sum_{h = 115\text{ mm}}^{185\text{ mm}} N_{y,h} \cdot e_h.$$

The scallop shell height size ranges for ages 2, 3, and 4 were obtained from visually evident height–frequency peaks in the survey data. Above age 4, when peaks are no longer distinguishable, a more approximate scale was derived from the age–height formula, $h(a)$, of Serchuk et al. (1979):

$$h(a) = 152.46\,\{1 - \exp[-0.3374(a - 1.4544)]\}.$$

Lognormal Recruitment

By definition, recruitment, R, is lognormal if the distribution of ln(R) is normal. Shapiro and Wilk's test (as coded by the NAG FORTRAN Library, Mark 13) quantifies normality. Recruitment variability for most regions is substantially more normal under log transformation (Table 1). For the largest population, on the Northern Edge and Northeast Peak (Fig. 3 and 4), the histogram of 10 ln(R) values, illustrated in Fig. 4, is well approximated by the sampled normal probability density function, implying that R (Fig. 3) varies lognormally. The {ln(R)} probability density function of Fig. 4 does not differ from normal (significance = 0.5), and there is a general increase by 2–4 orders of magnitude in Shapiro and Wilk's statistic for the log-transformed versus untransformed R-distributions in most regions (Table 1). The exceptions were the Southeast Part and possibly also the eastern South Channel where the densities of scallop recruitment were lowest, an order of magnitude less than the Northeast Peak and nearly 2 orders less than the Northern Edge (McGarvey et al. 1992). In addition, because the Georges Bank scallop population has been cycling (McGarvey 1991), the normalities of distributions of S are also modestly improved by log transformation.

To estimate the significance of stock–recruitment correlations, the lognormal distribution of recruitment must be explicit. A Monte Carlo routine was written to estimate the probability

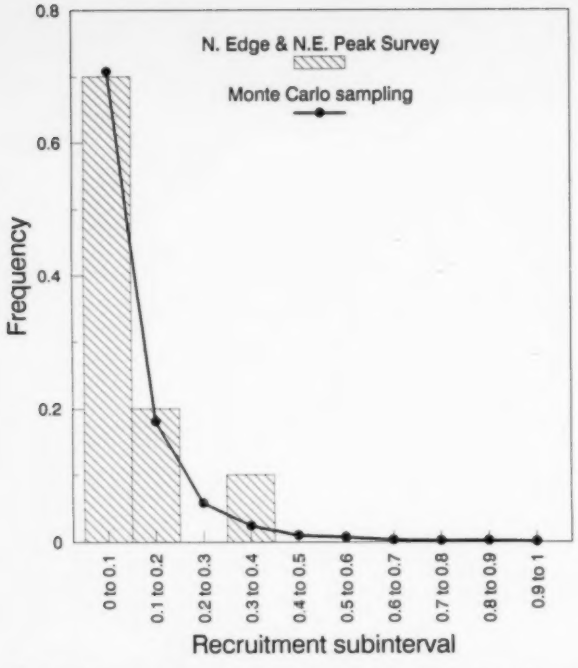

FIG. 3. Histogram of recruitment from the Northern Edge and Northeast Peak, 1977–86 (bars), compared with the sampled lognormal (line), obtained as the exponential of the normal Monte Carlo histogram plotted in Fig. 4.

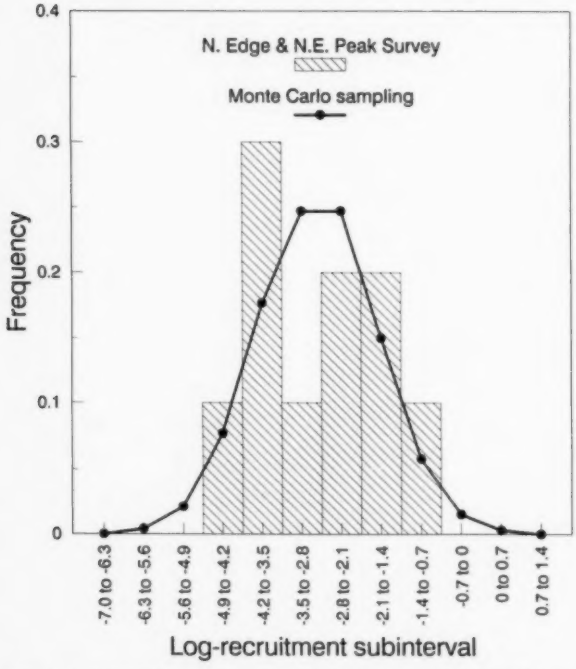

FIG. 4. Log-transformed recruitment distribution of Fig. 3 (bars) from which are taken the mean and variance for the Monte Carlo histogram (line) of 10 000 normally distributed test $\ln(R)$ values.

of obtaining the observed level of correlation by chance. A Monte Carlo test R-variate was generated from a lognormal distribution with a mean and a standard deviation identical to the observed $\ln(R)$-distribution in each subregion. Specifically, 10 $\ln(R)$ values were randomly drawn from their normal distribution (e.g. Fig. 4) using a standard two-random-number sampling algorithm (Abramowitz and Stegun 1970) and back-transformed, by exponential, to obtain 10 nonautocorrelated lognormally distributed test values of sampled recruitment (Fig. 3). The Monte Carlo S-variate was assigned the set of S values directly from observed data. Any effect that an unusual distribution of S might have on estimating significance is thereby explicit. Each sample of Monte Carlo recruit numbers, $\{R_{MC_y}\}$, yields a random series to correlate with the observed $\{S_y\}$ or $\{S5+_y\}$ time series. The frequency with which the Monte Carlo correlation exceeded the observed stock–recruitment correlation in 1000 trials yielded a P-value that the observed correlation occurred spuriously under the null hypothesis of no stock–recruitment relationship.

Since each subregion has a different mean and standard deviation of $\ln(R)$ and a different set of S-values, a separate Monte Carlo calculation was run for each. The only assumption is that the log of R be normally distributed. To that extent, quantified in Table 1, this Monte Carlo method gives a conventional estimate of P, with a precision limited by computer time. The standard error of each P-estimate, for 1000 runs, can be calculated analytically, P being the outcome of a random binomial process, as $\mathrm{SE}(P) = \sqrt{P(1-P)/1000}$. For the case of the Northern Edge and Northeast Peak, the Monte Carlo $P = 0.045$ yields a standard error of 0.0066, about $\pm 15\%$.

Results

Stock–Recruitment

Age of parent stock

Using $S4+$ in place of S yielded unexpectedly large increases in the stock–recruitment correlation (Tables 2–4). $S5+$ yielded another substantial increase. However, for a considerable number of cases, correlations with $S6+$ declined. The large increases between and within the Northern Edge and Northeast Peak, supposing that scallops aged 3 and 4 do not contribute viable eggs (Fig. 5), were exhibited to a smaller extent by the other subregions and Georges Bank as a whole. It was equally evident when the 1978 data point was omitted. Overall, this improvement was observed in every case when a stock–recruitment relationship was already in evidence, with or without 1978.

Within subregions

Because 84% of the scallops are found on the Northern Edge and Northeast Peak (McGarvey et al. 1992), the aggregated results for Georges Bank predominantly reflect the reproductive dynamics of this subregion. The correlations (Table 2; Fig. 5a) (or equivalently, the t-value of the slope, $t(b) = 2.57$) imply that recruitment on the Northern Edge and Northeast Peak tends to be higher in years when larger populations of adult scallops produce more eggs. This is additionally substantiated by the y-intercept, which passes close to the origin ($t(a) = -0.03$), as predicted for the stock–recruitment relationship of a heavily fished population, since well below the carrying capacity, extrapolating to zero eggs should tend to yield zero recruits.

The high negative correlation between stock size and recruit-

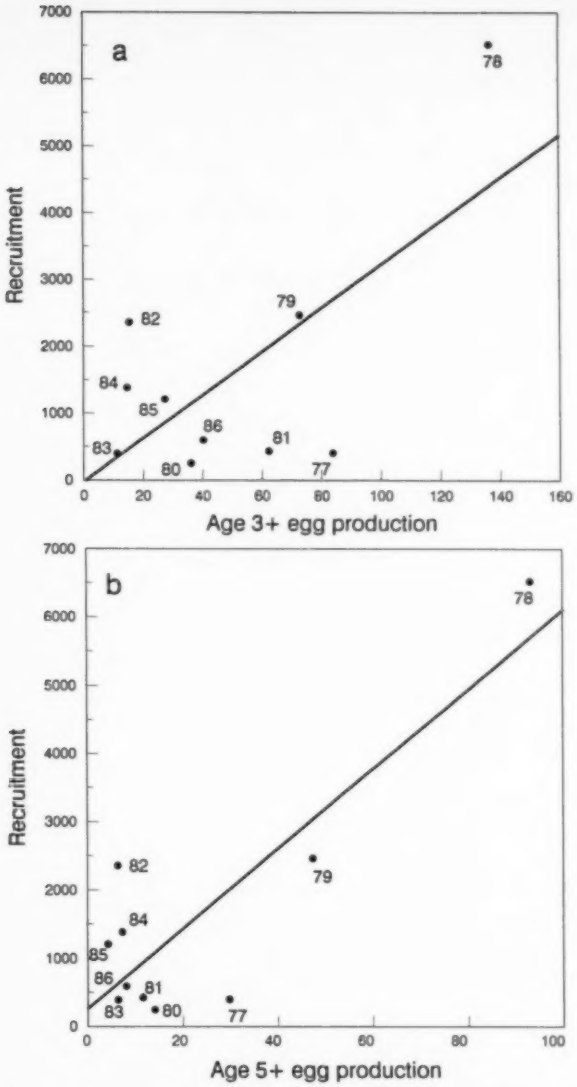

FIG. 5. Regressions of annual recruitment (10^6 2-yr-olds) versus egg production (10^{15} eggs) for the combined area of the Northern Edge and Northeast Peak using (a) S, the estimate of yearly egg production derived directly from the field measurements of MacDonald and Thompson (1985b), and (b) $S5+$, including only eggs from scallops above 105 mm, roughly age 5.

ment in the Great South Channel (Table 2) suggests that there is no stock–recruitment relationship within this region and may imply density dependence.

Between subregions

Stock–recruitment correlations between neighboring regions are presented in Tables 3 and 4. Two trends are evident. First, recruitment into the Northern Edge, the Northeast Peak, and the Southeast Part is best correlated with egg production originating in the Northeast Peak. Second, egg production from the South Channel, the Northern Edge, and the Northeast Peak is best correlated with recruitment into the Northern Edge. This may seem contrary to expectation, since the residual currents,

TABLE 2. Stock–recruitment correlations within Georges Bank and the three major subregions. The Monte Carlo estimation of significance is in brackets. In the Southeast Part, where recruitment was normal, the standard tabulated P-value is in parentheses.

	r			
Region	R vs. S	R vs. $S4+$	R vs. $S5+$	R vs. $S6+$
Georges Bank	0.564 [0.077]	0.677 [0.042]	0.731 [0.029]	0.682 [0.038]
Northern Edge and Northeast Peak	0.671 [0.045]	0.805 [0.029]	0.860 [0.010]	0.848 [0.018]
Southeast Part	0.12 (0.365)	0.204 (0.286)	0.211 (0.278)	0.209 (0.280)
South Channel (both halves)	−0.576 [0.994]	−0.393 [0.908]	−0.344 [0.880]	−0.371 [0.883]

on average, flow from the Northern Edge onto the Northeast Peak and may be thought to carry eggs only in that direction. Part of the explanation of the first trend may lie in the fact that 4.5 times as many eggs are released from the Northeast Peak as from the Northern Edge (McGarvey et al. 1992) (11.5 times more than the Southeast Part), primarily because it encompasses 10.5 times as much area. Since most eggs are produced there, a better correlation of stock from the Northeast Peak with recruitment to all neighboring regions would be anticipated if there is, indeed, a relationship between egg production and recruitment. The second trend may be explained by the fact that recruitment density on the Northern Edge is 3.6 times higher than on the Northeast Peak and 5.5 times higher than averge (McGarvey et al. 1992). Spat settling in this region find a more favorable and thus more reliable habitat for survival, so recruitment to the Northern Edge would therefore presumably be better correlated with any subpopulation whose eggs regularly drift there. These differences in correlation (Table 3), which could imply egg transport throughout the Northeast Peak and Northern Edge, corroborate the absolute r-value (Table 2) which (for both S and $S5+$) suggests that this population is self-sustaining.

When 1978 is included, the evidence weakly supports a relationship between recruitment to the Southeast Part with egg production from the Northeast Peak (Table 4). When 1978 is omitted, the opposite conclusion is drawn. Since overall scallop density is more than an order of magnitude less than in the Northern Edge, it is not surprising that recruitment in the Southeast Part should be more erratic, yielding lower correlations with likely sources of egg production. No relationship is observed between Southeast Part egg production and South Channel recruitment (Table 4).

In summary, the stock–recruitment relationships become stronger as spatial consideration is restricted to the populations neighboring or including recruitment to the Northern Edge from egg production in the Northeast Peak. For the regions of (i) Georges Bank taken as a whole, (ii) the Northeast Peak, (iii) the Northern Edge, (iv) the Northeast Peak and Northern Edge combined, and (v) recruitment to the Northern Edge with egg production from the Northeast Peak, the correlations of recruitment with $S/S5+$ increase as (i) 0.564/0.731, (ii) 0.633/0.783, (iii) 0.540/0.866, (iv) 0.672/0.860, and (v) 0.793/0.915. Thus the clearest association between stock and recruitment appears in the regions of greatest, densest, and most consistent population. These are also the regions of heaviest fishing.

Lastly we consider the correlation of recruitment time series between neighboring subregions (Table 5). The correlation of

TABLE 3. Stock–recruitment correlations for various parent stock ages on the Northern Edge and Northeast Peak. Monte Carlo significance is in brackets.

S-region (from which eggs originate)	*R*-region (into which spat settle and survive to age 2)	*r*			
		R vs. *S*	*R* vs. *S*4+	*R* vs. *S*5+	*R* vs. *S*6+
Northern Edge	Northern Edge	0.540 [0.10]	0.807 [0.015]	0.866 [0.007]	0.836 [0.010]
Northern Edge	Northeast Peak	0.424 [0.148]	0.686 [0.051]	0.746 [0.033]	0.719 [0.040]
Northeast Peak	Northern Edge	0.793 [0.016]	0.877 [0.007]	0.915 [0.004]	0.900 [0.006]
Northeast Peak	Northeast Peak	0.633 [0.051]	0.736 [0.038]	0.783 [0.027]	0.767 [0.029]

TABLE 4. Stock–recruitment correlations between neighboring regions of the Southeast Part and the South Channel. Monte Carlo significance is in brackets. The standard tabulated *P*-value is in parentheses.

S-region (from which eggs originate)	*R*-region (into which spat settle and survive to age 2)	*r*			
		R vs. *S*	*R* vs. *S*4+	*R* vs. *S*5+	*R* vs. *S*6+
Northern Edge	Southeast Part	0.321 (0.18)	0.420 (0.11)	0.420 (0.11)	0.374 (0.14)
Northeast Peak	Southeast Part	0.423 (0.11)	0.421 (0.11)	0.450 (0.10)	0.423 (0.11)
Northern Edge and Northeast Peak	Southeast Part	0.415 (0.12)	0.428 (0.11)	0.450 (0.10)	0.418 (0.11)
Southeast Part	Eastern half of South Channel	−0.437 [0.92]	−0.502 [0.96]	−0.507 [0.98]	−0.514 [0.98]
Southeast Part	Western half of South Channel	0.095 [0.39]	−0.052 [0.51]	−0.078 [0.54]	−0.074 [0.52]
Eastern half of South Channel	Eastern half of South Channel	−0.351 [0.92]	−0.379 [0.94]	−0.379 [0.94]	−0.379 [0.94]
Western half of South Channel	Western half of South Channel	−0.591 [0.99]	−0.408 [0.91]	−0.308 [0.83]	−0.294 [0.82]
Western half of South Channel	Eastern half of South Channel	−0.450 [0.95]	−0.475 [0.94]	−0.619 [0.994]	−0.682 [0.999]
Eastern half of South Channel	Western half of South Channel	−0.268 [0.82]	−0.249 [0.77]	−0.224 [0.73]	−0.270 [0.81]
Eastern half of South Channel	Northern Edge	0.269 [0.20]	0.293 [0.19]	0.381 [0.16]	0.251 [0.21]
Western half of South Channel	Northern Edge	0.111 [0.33]	0.293 [0.16]	0.419 [0.11]	0.191 [0.27]

recruitment on the Northern Edge with recruitment in the Northeast Peak ($r = 0.846$) is lower than with age 5+ egg production from the Northeast Peak ($r = 0.915$). If these two subregions share comparable environmental conditions and egg supply, this rough similarity in correlation suggests that yearly recruitment in this population is about equally influenced by egg production and environment. In these two subregions combined, where $r^2 = 0.860^2 = 0.74$ (Table 2), age 5+ egg production explains 74% of the variation in recruitment.

The overall variability of recruitment is observed to be considerable in all cases, typical of marine populations. For all subregions and for Georges Bank as a whole, the standard deviation of $\{R_y\}$ was greater than the mean, typically by about 20%. And in all cases except the Southeast Part and eastern South Channel, the autocorrelation of $\{R_y\}$ was negative or near zero between successive years (Table 6).

Discussion

The observed recruitment distribution is compatible with the model (1). Mathematically, a lognormal distribution results from a *product* of independent normally distributed random variables. Since recruitment each year is the product of intermediate survival probabilities from egg to age 2 (Walters and Hilborn 1976; Peterman 1978), lognormal recruitment may be likely for a species subject to wide annual variability at several stages of its development, particularly if population density is low. Hennemuth et al. (1980) found that annual recruitment variability

TABLE 5. Recruitment correlations between neighboring subregions.

		r
Northern Edge	Northeast Peak	0.846
Northeast Peak	Southeast Part	0.554
Southeast Part	South Channel (both halves)	0.246
Southeast Part	Eastern half of South Channel	0.330
Southeast Part	Western half of South Channel	0.234
South Channel (both halves)	Northern Edge	0.181
Eastern half of South Channel	Northern Edge	−0.100
Western half of South Channel	Northern Edge	0.190
Eastern half of South Channel	Western half of South Channel	0.402

was better described by a lognormal distribution in 15 of 18 fish stocks, and (Peterman 1981) in 11 of 12 salmon stocks. Total egg production is one of several independent multiplicative factors which change each spawning season, all of which determine the number of survivors to age of recruitment.

The years 1978 and 1979 dominate the regressions (Fig. 5). Characteristic of lognormal stochastic processes, the *tail* of the recruitment distribution is critical. For fish populations, large year classes often represent much of overall catch averaged over a number of years (Hennemuth et al. 1980; Sissenwine 1984; Rothschild 1986). We therefore ask whether it is coincidence that the two largest year classes in recent history occurred during a peak in scallop egg production. We consider four aspects of this question:

(1) Invoking model (1), we divide recruits each year by eggs spawned to obtain a time series of average egg to recruit survival. This measure of recruitment success due to factors besides egg production primarily reflects the environment of early life stage scallops, being largely density independent in heavily exploited populations. By this measure, 1978 was not a notable year; 1982 and 1984 were more environmentally favorable. In the combined area of the Northern Edge and Northeast Peak, $R_{1982}/S_{1982} = 1.5 \times 10^{-7}$ and $R_{1984}/S_{1984} = 9.5 \times 10^{-8}$, compared with $R_{1978}/S_{1978} = 4.7 \times 10^{-8}$. The trend of R/S for the Northern Edge, Northeast Peak, and Southeast Part is towards lower values around 1977–81 and towards considerably higher values around 1982–84.

(2) Comparing egg production, S, and environmental favorableness reflected in the survival rate per egg, R/S, with their respective mean values allows comparison of their relative contributions to the large 1978 year class. That year, R/S was 1.08 times higher, while total egg production was 2.67 times higher than average. This, together with model (1), implies that most of that year's recruitment success is explained by the greater production of eggs, due to an older and larger scallop stock (Fig. 2).

(3) Considering egg production by scallops age 5 and older, these ratios of $R/S5+$ and $S5+$ compared with their means are 0.60 and 3.81, respectively. If younger scallops are less reproductively mature, this would imply that factors besides egg production were relatively unfavorable in 1978 and that greater egg supply produced by a substantially older 1978 parent stock was the life history factor favoring record recruitment.

(4) The distortion of significance that would have resulted from these two extreme data points was removed by log transformation. The warnings of statisticians disallowing standard P-values for lognormal variates were certainly confirmed. As the correlation increases, the overestimation of significance by standard P-value tables also increases. For values of r around 0.8, the normal P-value is an order of magnitude overly significant, and for $r = 0.915$, it is $P = 0.0001$ (df = 10), implying a correlation 40 times more unlikely than the lognormal-variate Monte Carlo estimate $P = 0.004$ calculated here (Table 3).

If recruitment is lognormal due to the multiplicative nature of early life history survival, it is anticipated that poor recruitment will occur in some years of high egg production because conditions may have been poor at any other life history stage. Less likely is to find the highest levels of recruitment in years when any of the critical stages are unfavorable, including years of low egg production or unfavorable current patterns. In the Northern Edge and Northeast Peak, in no year when egg production was relatively low was recruitment high; in like manner, high recruitment only occurred in years of high egg production.

If the ratio of maximum to minimum egg production is considerably less than the ratio of maximum to minimum recruitment, then even when higher egg production does yield proportionally higher recruitment, this relationship may be unreflected in the stock–recruitment correlation. In the western South Channel, recruitment varies by a ratio of 28, while S and

TABLE 6. Variation of recruitment and stock variables from all subregions.

	Ratio of maximum to minimum recorded values, 1977–88			Autocorrelations between successive years		
Region	R	S	$S5+$	R	S	$S5+$
Georges Bank	17.2	6.3	7.1	−0.252	0.721	0.673
Northern Edge and Northeast Peak	26.1	12.2	21.8	−0.083	0.601	0.543
Northern Edge	14.5	14.0	27.3	0.024	0.676	0.625
Northeast Peak	16.7	11.7	20.2	−0.256	0.504	0.521
Southeast Part	33.9	4.35	6.6	0.109	0.737	0.748
South Channel (both halves)	27.9	4.0	7.1	−0.202	0.239	0.591
Eastern half of South Channel	27.5	7.4	10.9	0.443	0.436	0.529
Western half of South Channel	27.7	3.8	6.3	−0.215	−0.122	0.505

$S5+$ vary by ratios of 4 and 6 (Table 6). The remaining variation must be due to causes independent of egg production. In the Northern Edge and Northeast Peak, recruitment varies by the similar ratio of 26, while S and $S5+$ vary over factors of 12 and 22. This threefold wider $S/S5+$ variation would considerably enhance the chance of detecting a stock–recruitment relationship in the Northern Edge and Northeast Peak, allowing the possibility that in the western South Channel recruitment varies with egg production but that environmental variation is relatively greater.

The 1977 recruitment value, counted in the 1979 survey, may have been significantly underestimated because of the early timing of the cruise in May–June rather than August–September as in all subsequent surveys. Most growth occurs in summer. Correcting for bias due to lower sampling rate of these smaller juveniles would have resulted in slightly higher stock–recruitment correlations on the Northern Edge and Northeast Peak, since 1977 was a year of higher than average egg production.

Two previous scallop studies have reported evidence of stock–recruitment relationships. Dredge (1988) found some evidence of recruitment overfishing in the Australian saucer scallop trawl fishery. Caddy (1989) noted the production of abundant spat by the dense spawning population as a factor in the success of scallop culture operations in Mutsu Bay, Japan, which produce yields comparable to the Northern Edge and Northeast Peak on less than 10% of the surface area (Aoyama 1989). In corroboration, the graph of Aoyama (1989, fig. 10) exhibited an increasing relationship between net production and population density.

One potentially important difference in our method from previous studies (Mohn et al. 1989) was the construction of R and S time series directly from survey data, without intervening stages of ageing or virtual population analysis (VPA). Recruitment was obtained by summing numbers per tow over sizes 30–60 mm under the visually evident first peak (Fig. 2). Likewise, total egg production was estimated without assumptions about the age of sampled individuals by employing a fecundity vector with size as the independent variable. (Although not crucial, age assumptions were employed in estimating where to begin the sum in the calculation of $S4+$, $S5+$, and $S6+$.) Ageing introduces error when sampled individuals are misplaced into age classes above or below their correct cohort, reducing the precision of the resulting R and S time series. Large recruitment year classes are particularly subject to "peak-smearing." This can be exacerbated by mathematical smoothing (Bradford 1991) and uncertain assumptions about natural mortality (Sims 1984; Laponte et al. 1989) inside VPA.

We may summarize the evidence for a stock–recruitment relationship on the Northern Edge and Northeast Peak as follows:

(1) The stock–recruitment correlations themselves are statistically significant, despite lognormality. The year of highest egg production yielded highest recruitment. The year of second highest $S5+$ and third highest S egg production yielded second highest recruitment. No years of low egg production yielded high recruitment.

(2) The y-intercepts pass close to the origin.

The spatial pattern of correlations was also consistent with the hypothesis of spawn dispersal throughout the Northern Edge and Northeast Peak and a positive dependence of recruitment on egg production:

(3) Populations in regions of highest recruitment density (e.g. the Northern Edge) exhibited the strongest correlations with neighboring likely sources of egg production.

(4) Populations of highest absolute egg production (e.g. the Northeast Peak) were best correlated with recruitment to neighboring regions.

(5) Overall, for Georges Bank, the stock–recruitment correlations were highest where scallops were most abundant and recruitment most successful and thus where fishing was most intense, with the notable exception of the South Channel.

(6) The consistent increases in correlation observed when eggs from younger scallops were excluded, for essentially every case where a positive correlation was evident, may also implicate a relationship between eggs and recruits: a better measure of egg production should only improve correlations if there is a real underlying relationship between egg production and recruitment success.

(7) These increases from (R,S) to $(R,S5+)$ also follow the spatial distribution pattern predicted by a stock–recruitment relationship. Correlations increased most within and between regions where (R,S) correlations were highest.

(8) A lognormal distribution is consistent with the recruitment model (1), egg production being one of several important multiplicative factors of annual variability.

Egg Viability

Perhaps our most important result is the correlation evidence that 3- and, to a lesser degree, 4-yr-old scallops in these stocks may not contribute measurably to recruitment. In the Canadian fishery, scallops become large enough to harvest at an age of around 3.25 yr, at a size of about 80–90 mm (Mohn et al. 1989, tables 3 and 5). By 5.0 yr of age (measured from the time of spawning) the cohorts are typically reduced to less than 10% and by age 6 less than 1% of age 3.0 abundance (Mohn et al. 1989, tables 6 and 7). If an independent experiment could be undertaken to measure the relative viability and fertilization rate of eggs spawned by scallops of various ages, possibly comparing age 3 ($<\sim$90 mm) with larger scallops age 5 or older ($>$105 mm), the result could be of interest for both aquaculture and the natural fishery. At around age 5, scallops undergo fundamental changes in swimming behavior (Caddy 1968), susceptibility to predation (Elner and Jamieson 1979; Jamieson et al. 1982), and disposition on the bottom (Caddy 1968), which could affect fertilization. Strong indication of changes in production energy allocation may indicate that the reproductive physiology is still under development until age 5 when production is shifted from soma growth to gonad biomass released in spawning (MacDonald and Thompson 1985b, 1986b, 1988).

If scallops produce few viable spawn before age 5, the total egg production of the Georges Bank population is a small fraction of what it would be if they lived a natural life span of 20 yr or, more relevant for management, if intensive harvesting were delayed until scallops reached age 5 or 6. The one factor that could diminish the strength of this conclusion is the inhibiting effect on recruitment of density dependence. The results presented here, and a more detailed analysis (in preparation), suggest that density dependence is real but secondary and discernible only by comparison with a natural, unfished population.

At least three different yield per recruit investigations (Posgay 1962; Serchuk et al. 1979; Sinclair et al. 1985) have concluded that raising the average age of captured scallops would increase yields. A 2.9 factor of increase in lifetime fecundity per recruit was estimated if harvest were delayed from age 4

until age 6 (McGarvey et al. 1992) by gear or stricter meat-count size regulation. If scallops of age 3 and 4 are not fully mature, the estimated increase in egg production would be greater than 2.9. The correlations presented here raise the possibility that average recruitment could be comparably enhanced by raising age at first capture.

Acknowledgements

This work was supported by the Northeast Fisheries Center, U.S. National Marine Fisheries Service, Woods Hole, Massachusetts, and the Marine Fish Division, Bedford Institute of Oceanography, and the Benthic Fisheries and Aquaculture Division, Halifax Laboratory, of the Canadian Department of Fisheries and Oceans. Statistical consultation was provided by David Hamilton with the Statistical Consulting Service of Dalhousie University.

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Brown Trout (*Salmo trutta*) Status and Chemistry from the Norwegian Thousand Lake Survey: Statistical Analysis

Arthur J. Bulger

Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903, USA

Leif Lien

Norwegian Institute for Water Research, P.O. Box 69 Korsvoll, N-0808 Oslo 8, Norway

B. J. Cosby

Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903, USA

and Arne Henriksen

Norwegian Institute for Water Research, P.O. Box 69 Korsvoll, N-0808 Oslo 8, Norway

Bulger, A. J., L. Lien, B. J. Cosby, and A. Henriksen. 1993. Brown trout (*Salmo trutta*) status and chemistry from the Norwegian Thousand Lake Survey: statistical analysis. Can. J. Fish. Aquat. Sci. 50: 575–585.

Substantial negative impacts of acidic precipitation on freshwater fishes have been recorded over the last 50 yr in Norway. We analyzed the present status and historical trends of brown trout (*Salmo trutta*) populations in relation to water chemistry in 584 Norwegian lakes. Since the 1940s, 39% of the lakes have lost their trout populations, and another 17% of the lakes have declining trout populations; thus, 56% of the populations have been adversely affected. The results of analysis of variance, principal components analysis, and discriminant analyses converge on the variables pH, monomeric inorganic aluminum, and alkalinity as most strongly related to trout status. Lakes whose trout populations are extinct have an average aluminum concentration of 133 μg/L, an alkalinity of −34 μeq/L, and a pH of 4.8 versus 11 μg Al/L, +27 μeq/L, and 6.0 for lakes with healthy populations. Also, nitrate and sulfate levels are significantly higher in affected versus unaffected lakes. Calcium and sulfate appear to influence trout status moderately as individual variables, but strongly in combination, since they are the major determinants of alkalinity in these lakes. Models based on discriminant analyses showed good success (up to 89%) in predicting trout population status categories from water chemistry.

Au cours des cinquante dernières années, on a constaté que les pluies acides avaient eu des répercussions défavorables substantielles sur les poissons d'eau douce en Norvège. Nous avons analysé l'état actuel et les tendances historiques de la population de truites brunes (*Salmo trutta*) en fonction des caractéristiques chimiques de l'eau dans 584 lacs norvégiens. Depuis les années 40, 39 % des lacs ont perdu leur population de truites brunes et 17 % ont une population de truites en déclin. Ainsi, 56 % des populations ont été touchées défavorablement. Les résultats de l'analyse de variance, de l'analyse des composantes principales et des analyses discriminantes convergent pour indiquer que les variables qui influent le plus sur l'état des truites sont le pH, l'aluminium organique monomère et l'alcalinité. Les lacs qui ont perdu leur population de truites ont des valeurs moyennes de 133 μg/L pour la concentration d'aluminium, de −34 μéq/L pour l'alcalinité et de 4,8 pour le pH, comparativement à des valeurs respectives de 11 μg Al/L, +27 μéq/L et 6,0 dans les cas des lacs où la population se porte bien. De plus, la concentration de nitrate et de sulfate est substantiellement plus élevée dans les lacs touchés par les pluies acides. En tant que variables individuelles, le calcium et le sulfate semblent avoir en effet modéré sur la population de truites, mais leur effet combiné est très important puisque qu'ils sont les principaux constituants qui déterminent l'alcalinité de ces lacs. Des modèles fondés sur les analyses discriminantes montrent que l'on peut prévoir avec beaucoup de succès (jusqu'à 89 % l'état de la population de truites à partir des caractéristiques chimiques de l'eau.

Received February 28, 1991
Accepted September 22, 1992
(JA922)

Reçu le 28 février 1991
Accepté le 22 septembre 1992

A survey of 1005 lakes located throughout Norway was conducted in 1986 (Henriksen et al. 1988) to establish a reference database prior to major planned reductions in atmospheric emissions of acidic precursors in Europe; the survey was also designed to compare the 1986 status of lakes with the results of extensive regional surveys made in 1974–75. These two large studies documented extensive lake acidification in an area of 36 000 km^2 associated with severe damage to fish populations, including the extinction of hundreds of local populations. Furthermore, the number of barren lakes doubled in southernmost and southwestern Norway between 1974–75 and 1986 (Henriksen et al. 1989). Previous reports based on these surveys have described present and predicted future status of lakes in Norway (Henriksen et al. 1988; Hornberger et al. 1989; Wright et al. 1991) and present and predicted future freshwater fish community status (Henriksen

et al. 1989). The second of these (Henriksen et al. 1989) dealt with the relationship between water chemistry and a variable called General Fish Status, an index designed to reflect the condition of the fish community in lakes, whether composed of single or multiple species.

Here we focus on the relationships between water chemistry and the status of a single species, brown trout (*Salmo trutta*). The brown trout is Norway's most economically important freshwater fish, and considerable information spanning several decades exists on the density and population structure for many brown trout populations. Our goals were to (1) identify the chemical parameters that show the strongest relationship to brown trout population status, (2) characterize chemically lakes classified by trout status, and (3) develop statistical models that predict brown trout status from water chemistry. Principal components analysis and analysis of variance were used to address the first two goals, and discriminant and regression analyses were used to address the third.

Methods

Data Set and Variables

The information on chemistry and trout status in this report came from the Thousand Lake Survey (Henriksen et al. 1988). The chemical data were obtained using standard laboratory methods (Henriksen et al. 1988). Information on chemistry, the present status, and the change in status of brown trout populations was available for 584 lakes, all of which had brown trout populations in the 1940s. None of these lakes has been stocked with fish, limed, or treated with rotenone; none has had any significant water level regulation, local pollution source, or disturbance to spawning substrate. None of the lakes have been used for hydroelectricity generation; in short, lakes with any significant human influence other than fishing were avoided. The lakes are located in bedrock areas expected to yield low ionic strength surface waters (roughly 45% of the surface area of Norway is underlain by such bedrock); the lakes are distributed throughout Norway, but the majority are located in the southern half of the country. The fish status information, in the form of interviews, was collected by local fisheries authorities in each of Norway's counties and then verified by fisheries experts at the Norwegian Institute for Water Research (NIVA) and elsewhere (Henriksen et al. 1989). Although the criteria for the fish status categories are somewhat subjective (see below), the system is easy to use and has established its utility over the past several decades; it also makes possible invaluable historical comparisons. More than 50 of the lakes in this report were test-fished as well (some on multiple occasions spanning years to decades) using a standard gillnet sampling procedure (Rosseland et al. 1980), which included nets of eight different mesh sizes connected in a 27-m-long, 1.5-m-high curtain; multiple curtains were employed simultaneously in each lake. In this and other brown trout surveys in Norway (Wright and Snekvik 1978; Sevaldrud and Skogheim 1986), agreement between testfishing results and interviews exceeded 90% e.g. none or very few fish are caught in lakes reported to be barren, many fish are caught in lakes reported to be healthy, and marginal lakes produce intermediate numbers of fish.

This article will focus on two categorical trout status variables that were given integer scores: Trout Status and Change in Trout Status, defined as follows.

Trout Status: The condition of a lake's trout population at the time of the Thousand Lake Survey in 1986 (updated for a few lakes in 1990); three levels are scored (Rosseland et al. 1980):

(1) Healthy: successful recruitment common, as evidenced by the presence of many year classes and normal population structure; density of fish high enough to support satisfactory yield. When testfished, catch-per-unit-effort is greater than 1 fish per mesh size per night, but usually higher.

(2) Marginal: sparse population, either historically so, or reduced by acidic deposition; density of fish too low to support practical exploitation. Historically sparse populations typically have many year classes but low density; in populations damaged by acid deposition, recruitment failure is common, as evidenced by the presence of only a few year classes, as well as low density. When testfished, catch-per-unit-effort is between 0 and 1 fish per mesh size per night.

(3) Extinct: population lost since the 1940s apparently due to acidic deposition.

Change in Trout Status: The change (if any) in the status of a lake's trout population between the 1940s and 1986 (updated for a few lakes in 1990); four levels are scored for analysis:

(1) Improved: yield, recruitment success (number of year classes) and/or density of population has increased since the 1940s.

(2) Unchanged: no significant change in yield, recruitment, and/or density since the 1940s.

(3) Declined: yield, recruitment, and/or density reduced since the 1940s, apparently by acidic deposition.

(4) Extinct: population lost since the 1940s apparently due to acidic deposition.

These trout status variables were analyzed with respect to the following chemical variables: major ions (Ca, Mg, Na, K, Cl, SO_4, NO_3) (microequivalents per litre), ANC (acid-neutralizing capacity) (microequivalents per litre), calculated from measured variables as ANC = (Ca + Mg + Na + K) − (Cl + SO_4 + NO_3), N-M SO_4 (nonmarine/excess sulfate) (microequivalents per litre), pH, TOC (total organic carbon) (milligrams per litre), Al_a (total monomeric aluminum) (micrograms per litre), Al_o (organically bound monomeric aluminum) (micrograms per litre), and Al_i (inorganic monomeric aluminum, $Al_i = Al_a - Al_o$) (micrograms per litre). Aluminum fractions are operationally defined (Driscoll 1984; Henriksen et al. 1989). Because ANC is a linear combination of the seven major ions, ANC and the seven major ions were not used together as independent variables in any individual multiple regression; likewise, only two of the three aluminum variables were used in any individual analysis.

Statistical Methods

All analyses were performed with the statistical packages StatView II™ or SPSS™.

Principal components analysis (PCA)

PCA recognizes and quantifies the intercorrelations among the original variables in a data set. It is the type of factor analysis of choice when the goal is to reduce the dimensionality of a data set to a small number of new variables (principal components) which explain most of the variance in the data set. The principal components can be represented by a set of mutually perpendicular axes constituting a multidimensional space; rotation of these axes is commonly used to maximize the variance explained along each axis and to improve the interpretability and scientific utility of the results, without changing the underlying mathematical properties (Comrey 1973; Tabachnik and

TABLE 1. Descriptive statistics for chemical variables (N = 584 lakes).

Variable	Mean	SD	Minimum	Maximum	Units
pH	5.43	0.69	4.32	7.08	
Al_i	70.67	71.87	−4.00	298.00	µg/L
Al_n	105.09	79.63	10.00	350.00	µg/L
Al_o	34.41	28.34	10.00	202.00	µg/L
ANC	−1.81	40.55	−94.84	188.21	µeq/L
Ca	47.58	32.51	6.49	226.05	µeq/L
NO_3	7.74	7.39	0.00	49.64	µeq/L
N-M SO_4	60.59	32.68	7.66	211.30	µeq/L
SO_4	69.08	35.81	12.48	232.96	µeq/L
K	5.82	4.07	0.77	35.84	µeq/L
Mg	28.05	20.02	3.92	126.74	µeq/L
Na	76.34	69.55	6.53	435.00	µeq/L
Cl	82.74	82.40	2.82	507.60	µeq/L
TOC	2.65	2.22	0.10	17.20	mg/L

Fidell 1989). There are two classes of rotation: orthogonal and oblique. In orthogonal rotation, the components remain uncorrelated with each other, and a loading matrix is produced (such as that in Table 3), which is a set of correlation coefficients between the original variables and the principal components. Varimax rotation, a type of orthogonal rotation, is the commonest rotation technique and is reported in the present analysis. Varimax rotated loadings, like correlation coefficients, can assume values between −1.0 and +1.0. An oblique rotation (not reported) was also applied to these data; in this instance, oblique rotation yielded the same suites of variables associated with components explaining very similar proportions of the total variance.

The inclusion of categorical variables (Trout Status and Change in Status) does not preclude the use of PCA. As long as PCA is used descriptively as a convenient way to summarize the relationships among a large set of observed variables, assumptions regarding the distributions of variables (e.g. normality) are not in force (Tabachnik and Fidell 1989).

Analysis of variance (ANOVA)

ANOVA ws used to compare chemical differences among lakes classified by the status of their trout populations. These analyses were performed on untransformed variables.

Discriminant analysis

Discriminant function analysis is designed to predict group membership from a set of variables. It is conceptually and mathematically related to ANOVA: group membership serves as the independent variable in ANOVA and the dependent variable in discriminant analysis. Variables are often referred to as "predictors" in discriminant analysis. The major question in discriminant analysis is whether combinations of predictors (called discriminant functions) can predict group membership reliably (Tabachnik and Fidell 1989). In the present instance, most of the measured chemical variables as well as the calculated variables ANC, N-M, SO_4, and Al_i were used in the stepwise discriminant analysis based on minimization of Wilk's lambda as a criterion for entering a variable into the analysis (Tatsuoka 1971).

The resultant discriminant functions were interpreted by calculating the matrix of pooled-within-group correlations between the discriminating variables and the scores of the canonical discriminant functions. The variables that have the largest correlations with a given function can be viewed as "defining" that function. The derived functions were used to reclassify each lake as a measure of robustness of the discriminant model. Comparing the numbers of actual and predicted lakes in a given class provides a measure of model goodness-of-fit, analogous to r in regression analysis.

TABLE 2. Contingency table for Trout Status (Status) and Change in Trout Status (Δ Status). For the purposes of analysis the status categories were assigned numeric codes, as shown in the table and explained in the text. Note that only six of the 12 cells contain observations.

	Status (numeric code)			
Δ Status	Healthy (1)	Marginal (2)	Extinct (3)	Total
Improved (1)	24	4	0	28
Unchanged (2)	166	64	0	230
Declined (3)	0	98	0	98
Extinct (4)	0	0	228	228
Total	190	166	228	584

Regression analysis

It is not strictly appropriate to use regression analysis with discontinuous y-variables (Status and Change in Status can assume only integer values of 1–3 and 1–4, respectively). However, we included regressions to compare the empirical relationships between lake chemistry and fish status observed in our data set with those of other studies that used regression with discontinuous fish status variables despite the violation of this assumption of regression analysis. Furthermore, it is clear from our results that the chemical variables are not truly independent variables (see Table 3); nevertheless, we used them in multiple regressions for the same purpose of comparison with earlier studies which used these chemical variables as though they were independent variables. We also compared the r-values obtained in our simple regressions with rho-values (rank order correlation coefficient, corrected for ties), a nonparametric analog to the correlation coefficient r; as expected for a data set of this size, the r- and rho-values were very similar, i.e. both analyses identified the same chemical variables as being strongly or weakly associated with trout status.

Results and Discussion

In general, the 584 lakes are low in ionic strength, pH, organic carbon content, and buffering capacity (ANC) (Table 1); most of their sulfate is of nonmarine origin, i.e. the result of atmospheric pollution. Populations classified as Healthy in 1986 (Table 2) have either Improved (n = 24) or remained Unchanged (n = 166) since the 1940s. Of the Marginal populations, 98 have declined since the 1940s, but 64 of the Marginal lakes have probably always had sparse populations. In many instances, these historically sparse populations appear to be limited by spawning substrate. A small number (four) of Marginal populations have Improved since the 1940s. Of the 584 lakes in the present study, 39% have lost their trout populations since the 1940s; thus the lakes whose populations have Declined (n = 98) or become Extinct (n = 228), apparently due to acid deposition, represent a majority (56%) of the study lakes.

Principal Components Analysis

PCA identified four components explaining nearly 100% of the variance associated with the original variables. The com-

TABLE 3. Principal components analysis. Eigenvalues, with proportion of original variance in parentheses, percent variance explained (rotated), and varimax rotated loadings (correlation coefficients) for Trout Status (Status), Change in Trout Status (Δ Status), and 12 chemical variables with respect to the four components extracted in the PCA. The highest correlation coefficient for each variable is shown in bold face; variables having their highest correlations on the same component are boxed. Variables with high coefficients for the same component are intercorrelated.

	Component			
	1	2	3	4
Eigenvalue:	4.872 (0.348)	4.431 (0.316)	2.053 (0.147)	1.038 (0.074)
% variance:	38%	29%	17%	16%
Variable				
Status	**0.879**	−0.172	0.056	0.054
Δ Status	**0.915**	−0.103	0.036	0.006
ANC (μeq/L)	**−0.888**	−0.201	0.196	0.177
pH	**−0.937**	−0.094	−0.118	0.137
Al_i (μg/L)	**0.890**	0.081	−0.006	0.141
Mg (μeq/L	−0.067	**0.868**	0.204	0.369
Na (μeq/L)	0.034	**0.976**	0.029	0.083
K (μeq/L)	−0.118	**0.647**	0.088	0.567
Cl (μeq/L)	0.070	**0.980**	−0.001	0.049
Al_o (μg/L)	0.082	0.106	**0.946**	0.088
TOC (mg/L)	−0.049	0.058	**0.951**	0.151
Ca (μeq/L)	−0.483	0.106	0.269	**0.786**
SO_4 (μeq/L)	0.320	0.434	0.267	**0.722**
NO_3 (μeq/L)	0.485	0.434	−0.306	**0.528**

ponents, together with the percentage of the variance explained by each, are characterized below. Recall that after orthogonal rotation, the values in the loading matrix (Table 3) are correlation coefficients between the original variables and the principal components (Comrey 1973; Tabachnik and Fidell 1989). The greater the loading (correlation), the stronger the association between the variable and principal component. Variables that have large loadings *of the same sign* for the same component are positively intercorrelated; variables that have large loadings *of the opposite sign* for the same component are negatively intercorrelated. By definition, variables with large loadings on one component are uncorrelated with variables having large loadings on a different component.

The primary goal of the PCA is to focus attention on the underlying processes which are responsible for most of the variance in the data set. In the following discussion, each component is given a name intended to describe the physical processes most likely responsible for the observed relationship; this material represents an interpretation of the relationships observed in the data. The percentage of the variance in the data set associated with each component is given in parentheses after the name. The most important theme in the data set for the present purpose is recognized in principal component 1.

Principal component 1

Trout Status/Lake Acidification component (38%). This component recognizes the chemical variables most strongly associated with the condition of trout populations. Both Trout Status and Change in Trout Status are highly negatively correlated with pH and ANC (Table 3). Lakes with high pH and alkalinity are likely to have Healthy trout populations (scored as "1") whereas lakes with low pH and ANC are more likely to have Marginal (scored as "2") or Extinct populations (scored as "3"). Trout Status and Change in Trout Status are positively correlated with Al_i because lakes with high concentrations of aluminum are more likely to have damaged populations, and thus higher scores for both trout status variables. The Al_i (labile, or inorganic monomeric aluminum; Driscoll 1984; Henriksen et al. 1989) fraction of the total aluminum in solution in lakes is the fraction that is usually considered the most toxic to fish (Driscoll et al. 1980; Rosseland et al. 1990).

The underlying processes that produce the strong association among the trout status variables and ANC, pH, and Al_i can be related to physical/chemical interactions and direct toxicological effects on fish. These complex processes will be considered in some detail because of their central role in explaining the statistical relationships which are the focus of this paper.

Trout population status is very much related to the concentration of hydrogen ion (pH) and Al_i because both are directly toxic to fish (Baker and Schofield 1980; Leivestad 1982), exerting their primary effects on electrolyte balance functions at the gill (Muniz and Leivestad 1980; Leivestad 1982; Rosseland et al. 1990). Both H and Al_i reduce the concentrations of plasma sodium and chloride (Wood 1989). The Al-induced plasma ion reduction reflects both an increased efflux and decreased influx of ions. The reduced influx is probably caused by lowered activity of gill regulatory enzymes (Rosseland et al. 1990, 1992). The Al-induced efflux, like the H-induced efflux, reflects an increase in permeability to plasma ions probably caused by weakening the tight junctions between gill cells (Marshall 1985). Under extreme conditions, the respiratory function of the gill may also be impaired. Aluminum, especially in experimental treatments as aluminum sulfate, can cause excess mucus production at the gill epithelium; this increases the diffusion distances for gas exchange, so that respiratory failure may contribute to mortality. It seems likely, however, that mortality in nature is more often caused by ionoregulatory failure (Rosseland et al. 1990).

The intercorrelation among ANC, pH, and Al_i is also readily explained. While aluminum is abundant in nature, it is relatively insoluble in the neutral pH range (6.0–8.0) and is thus relatively unavailable biologically. However, under acid conditions, the solubility of aluminum is enhanced (Driscoll and Schecher 1990). The pH-dependent distribution of aluminum among its chemical species in solution is a major factor influencing aluminum toxicity (Bertsch 1990). Simply stated, the toxicity of aluminum is pH dependent (Baker 1982; Leivestad 1982). ANC largely controls pH and therefore is related to both aluminum concentrations and fish population response.

Calcium directly ameliorates the physiological stresses to fish in acidified waters (Leivestad et al. 1980; Wood and McDonald 1982; 1987; Brown 1983). The ameliorating effect of calcium is thought to result from supporting the tight junctions between cells, thereby reducing the passive loss of ions (reviewed in Rosseland et al. 1990).

Calcium, sulfate, and nitrate have moderate loadings on the Trout Status/Lake Acidification component (component 1, Table 3: −0.483, 0.320, and 0.485, respectively). A moderate loading of opposite sign with respect to trout status indicates that Healthy trout populations tend to occur in lakes with relatively high calcium. Loadings of the same sign with respect to trout status indicate that as the concentrations of acidifying pollutants increase, the Trout Status score is more likely to be "3" (Extinct) than "1" (Healthy). It is reasonable to ask why the association between trout status and these variables is not stronger, given calcium's beneficial physiological effect and the role of sulfate and nitrate in acidifying lakes. The reason is that the response to acidifying pollutants is calcium dependent. Healthy populations may be found in lakes with high as well as quite low calcium concentrations (as low as 11 μeq/L in this study), provided sulfate is also low; further, high calcium concentrations in lakes with Declined populations (as high as 155 μeq/L in this study) may not protect fish where sulfate loads are excessive. Thus, calcium by itself would not be a good predictor of trout status.

An additional interpretation of this aspect of the relationship among calcium, sulfate, and trout status is that calcium is the dominant cation and sulfate is the dominant anion in these lakes; the difference in their relative concentrations is thus the major contributor to ANC and pH, which are strongly related to trout status. A stepwise multiple regression of ANC on its seven component ions indicated that calcium and sulfate account for 89% of the variance in ANC; the next most important variable is nitrate, which brings the percentage to 92%. Nitrate may be more strongly associated with trout status than sulfate (suggested by a higher loading of component 1, Table 3) because elevated levels of nitrate in lakes occur chiefly when atmospheric inputs exceed the capacity of the plants in the catchments to take up this nutrient, i.e. when acidification is well underway.

Principal component 2

Sea Salt component (29%). The most abundant ions in seawater are Cl, Na, Mg, and SO_4. The underlying processes that produce the strong positive intercorrelation among Mg, Na, and Cl which are recognized by this component are the cotransport and codeposition of these ions in sea salt (Table 3). Although sulfate has a seawater source, the variance in sulfate is largely uncoupled from the variance in sea salts because acidic deposition is the major source of sulfate to these lakes (Wright and Snekvik 1978; Reuss et al. 1987; Henriksen et al. 1988). Sulfate has only a moderate association with component 2 and is more strongly correlated with the Geochemical Response component (component 4, Table 3). Since the sea salt variables are associated with a different component, they are largely uncorrelated with the trout status variables (Table 3). Thus, among these low ionic strength lakes, the concentration of sea salts has little impact on trout populations. The mechanism for this may be in part that sodium and chloride are transported and deposited in a constant ratio and that their charges almost balance, having minimal impact on ANC, pH, and Al_i. Potassium has a seawater source as well, and has its strongest association with this component; however, it is also associated with the Geochemical Response component (4).

Principal component 3

Total Organic Carbon/Organically Bound Aluminum (17%). This Component recognizes the strong positive correlation between TOC and Al_o and that the concentration of this nontoxic fraction of total aluminum is uncorrelated with trout status in this study (Table 3). The TOC/Al_o correlation is the result of the physical/chemical processes by which aluminum can be bound to organic complexes. Humic materials can ameliorate the effects of acidification by binding aluminum and rendering it nontoxic (Baker and Schofield 1980; Driscoll et al. 1980; Henriksen et al. 1989; Rosseland et al. 1990), so the concentration of Al_o would not be expected to have a strong positive or negative relationship to trout status, and indeed the association of Al_o with a different component than the trout status variables suggests that this is the case. The concentration of TOC in these trout lakes is low (the mean for all 584 lakes in this data set is 2.65 mg/L; Table 1). By contrast, the perch (*Perca fluviatilis*) populations in the Thousand Lake Survey occur in lakes with higher TOC (up to 12 mg/L), and the relationship between healthy perch populations and high TOC is strong (Henriksen et al. 1989).

Principal component 4

Geochemical Response component (16%). This component recognizes a positive correlation among calcium, nitrate, and sulfate (Table 3). The primary explanation for this intercorrelation lies in the relationship in southern Norway between geologic and anthropogenic processes: the areas with the thickest soils, and hence most calcium available from weathering and exchange processes, are also the areas that receive the greatest loads of nitrate and sulfate via long-range transport (Wright and Snekvik 1978). Deeper soils and greater weathering and exchange result in a partial buffering of acidic deposition. That is, the increased atmospheric inputs of sulfate and nitrate to the soils are partially balanced by increased release of calcium from the soils, resulting in a positive correlation between calcium and sulfate/nitrate in drainage waters. Magnesium and potassium can also buffer incoming acids as reflected by their moderate loadings on this component. This geochemical buffering response is, however, only weakly related to fish status because it is the absence of buffering (with resultant weak or negative correlations between calcium and sulfate/nitrate) that is associated with decreases in pH and declines in fish populations.

Analysis of Variance

There are substantial differences in chemistry among lakes classified by Trout Status and Change in Trout Status (Table 4). Lakes that have lost their trout populations (Extinct) have the lowest ANC, pH, and calcium concentrations, together with the highest labile aluminum (Fisher's PLSD test, 0.05 level;

TABLE 4. Analysis of variance ($N = 584$ lakes). Lakes classified by Trout Status (Status) and Change in Trout Status (Δ Status), using the six cells from Table 2 as classifying categories. Shown are mean values and standard error of the mean for seven variables. All seven one-way ANOVAs were highly significant ($p < 0.0001$). Means that are not significantly different (Fisher's PLSD test; 0.05 level) are given the same superscript letter. Note the small sample size for Marginal Improved.

Status:		Marginal			Healthy	
Δ Status:	Extinct	Declined	Unchanged	Improved	Unchanged	Improved
n:	228	98	64	4	166	24
Variable						
ANC (μeq/L)	-33.98^{a}	-9.97^{b}	35.95^{c}	77.67^{d}	26.26^{e}	29.00^{cd}
	(1.43)	(2.43)	(4.88)	(29.2)	(2.43)	(5.47)
pH	4.84^{a}	5.19^{b}	6.06^{c}	6.48^{de}	6.03^{c}	6.08^{cd}
	(0.02)	(0.04)	(0.07)	(0.31)	(0.04)	(0.09)
Al_i (μg/L)	132.55^{a}	79.11^{b}	17.25^{c}	24.00^{c}	11.34^{c}	9.08^{c}
	(4.07)	(5.68)	(3.28)	(15.48)	(1.34)	(3.91)
NO_3 (μeq/L)	10.27^{a}	9.25^{ab}	4.21^{c}	2.95^{bc}	5.51^{c}	3.16^{c}
	(0.41)	(0.74)	(0.72)	(1.82)	(0.64)	(1)
Ca (μeq/L)	30.95^{a}	50.17^{b}	70.73^{c}	111.15^{d}	57.88^{e}	51.48^{be}
	(1.23)	(2.68)	(4.97)	(39.36)	(2.72)	(5.57)
Total SO_4 (μeq/L)	75.04^{a}	83.82^{b}	65.65^{ac}	68.64^{abcd}	56.47^{cd}	48.62^{d}
	(2.10)	(3.56)	(4.82)	(20.56)	(2.80)	(5.75)
N-M SO_4 (μeq/L)	67.07^{ab}	73.34^{a}	59.97^{b}	67.70^{abc}	47.22^{c}	39.77^{c}
	(1.81)	(3.20)	(4.65)	(20.42)	(2.56)	(5.71)

Table 4). These lakes have in fact 12 times as much toxic aluminum as Healthy lakes.

Among Marginal lakes, those in the Declined category differ significantly from Unchanged lakes for all variables, having lower ANC, pH, and calcium, together with higher labile aluminum, nitrate, and sulfate (Fisher's PLSD test, 0.05 level; Table 4). Marginal lakes that are in the Improved category will not be discussed in detail because the small sample size ($n = 4$) limits inferences that can be made. It seems clear that further deterioration in water quality will lead to the extinction of the native trout populations in at least some of the lakes in the Declined category.

Among Healthy lakes, Unchanged and Improved lakes do not differ significantly from each other in any of the water quality parameters. Healthy lakes (both Unchanged and Improved) and Marginal Unchanged lakes are thought to have had no significant impacts on their fish populations as of 1986, the time of the Thousand Lakes Survey. These three categories of unaffected lakes do not differ significantly from each other in pH, labile aluminum, or nitrate (Fisher's PLSD test, 0.05 level; Table 4). However, the Marginal Unchanged lakes do have significantly higher nonmarine sulfate than the Healthy lakes (both Unchanged and Improved), which may be compensated by significantly higher calcium, giving the Marginal Unchanged lakes a significantly higher ANC relative to Healthy lakes (Fisher's PLSD test, 0.05 level; Table 4). Because the Marginal Unchanged lakes have significantly better water quality than the Marginal Declined lakes, some other factor has limited these populations to low densities historically; minimal spawning substrate is probable in many instances.

The overall pattern revealed by ANOVA is that lakes that have lost their trout populations (Extinct) have significantly poorer water quality than the other categories; lakes in the Declined category have significantly better water quality than Extinct lakes, but still significantly poorer water quality for most variables than the unaffected lakes (Marginal Unchanged, Healthy Unchanged, and Healthy Improved). The picture for calcium and sulfate is complex: Marginal Unchanged lakes have intermediate sulfate levels, but more calcium than Healthy lakes (Table 4). Perhaps if this calcium were unavailable in these lakes, negative effects due to the elevated sulfate concentrations might have occurred.

The PCA and ANOVA results described above establish that strong relationships exist among lake chemical variables and trout response variables. We turn now to the goal of developing models for predicting trout response using discriminant and regression techniques.

Discriminant Analysis

The goal of discriminant analysis is to classify cases into one of several mutually exclusive groups, based on values of a set of predictor variables. The classification rule is developed on cases for which group membership is known, and the rule can be applied to cases for which group membership is unknown. A necessary condition for the development of discriminant functions that can differentiate among the classification groups is that some or all of the predictor variables must differ significantly among groups.

The preceding analyses have provided some prior knowledge of the degree to which predictor variables differ among groups of lakes classified by the trout response variables Trout Status and Change in Trout Status. The PCA results indicate that a high correlation exists between these two trout response variables, and the ANOVA results suggest that the six groups based on the original fish response variables (Table 2) are not all truly distinct. Therefore, before beginning the discriminant analysis, we defined categories for a new trout response variable based largely on statistically significant differences among categories.

The ANOVA results suggest that there are no substantive differences among chemical variables for Healthy Improved lakes versus Healthy Unchanged lakes. These two classes were collapsed into a single group for the discriminant analyses. Also, the small number of cases in the Marginal Improved category ($n = 4$) makes it extremely difficult to draw inferences regarding membership on this class; these four were added to the Marginal Unchanged group. With respect to the predictor variables, all measured variables were used with the exception that Al_i

TABLE 5. Discriminant analysis. *F*-statistics of significant variables included in the discriminant analysis, listed in descending order of magnitude.

Variable	*F*-statistic
pH	62.3
Al_i	22.9
Ca	7.0
Cl	4.6
TOC	3.4
K	3.2
Na	3.1
Mg	2.4
NO_3	1.9

(the calculated difference between Al_a and Al_o) was used instead of Al_a and Al_o. The calculated variables ANC and N-M SO_4 were also included in light of their importance in preceding analyses. A maximum of three discriminant functions was possible with this design.

The analysis identified all three discriminant functions as significant ($p < 0.0001$). The first function accounted for more than 72% of the explanatory power of the analysis (canonical correlation = 0.85), while the remaining two functions accounted for 9 and 7%, respectively (canonical correlations = 0.30 and 0.27). The relative importance of individual variables in the overall analysis can be understood by examining the *F*-statistic used to include or remove a variable from the analysis (Tatsuoka 1971). The larger the *F*-statistic, the larger the relative importance of the variable in discriminating among the classes. On this basis, pH, Al_i, and calcium are the most important variables for explaining class membership (Table 5). The variables ANC, SO_4, and N-M SO_4 were not included in the functions by the stepwise procedure.

The matrix of pooled-within-group correlations between the predictor variables and the scores of the canonical discriminant functions (Table 6) can be used to interpret the functions. The correlation of any variable with a given function can be considered the "loading" of that variable on the function. These loadings can be used in much the same way as loadings are used to define components in PCA. Note that even though a variable is not used in constructing the discriminant functions, its correlation with the function scores can be calculated (cf. ANC in Tables 5 and 6). Some variables from a set of intercorrelated variables may not be needed in a model to predict the response accurately.

The highest loadings on function 1 (which contains most of the explanatory power) are for pH, Al_i, and ANC (Table 6). The signs are consistent with previous interpretations of these variables on the Trout Status/Lake Acidification component. That is, pH and ANC increase or decrease together, and low pH/low ANC are associated with high Al_i values. This function also shows moderate correlations with calcium, nitrate, total sulfate, and nonmarine sulfate. The signs for the loadings for these ions are consistent with the concept that they are the main determinants of ANC in these lakes. The second and third discriminant functions, while containing relatively little explanatory power, can still be recognized from their loadings. The second function represents a geochemical response, and the third represents sea salts (both as in PCA results).

The robustness or goodness-of-fit of the disciminant model can be assessed by "predicting" group membership for each

TABLE 6. Discriminant analysis. Pooled-within-groups correlations between predictor variables and canonical discriminant functions. The highest correlation coefficient for each variable is shown in boldface; variables having their highest correlations on the same function are boxed.

	Correlation		
Variable	Function 1	Function 2	Function 3
pH	**0.89**	−0.02	0.38
Al_i	**−0.73**	0.18	0.24
ANC	**0.66**	−0.39	0.30
Ca	0.29	**−0.64**	0.16
N-M SO_4	−0.19	**−0.62**	−0.04
SO_4	−0.17	**−0.55**	−0.18
TOC	0.00	**−0.45**	−0.10
Cl	0.00	0.07	**−0.60**
Na	0.02	0.01	**−0.60**
K	0.09	−0.11	**−0.56**
Mg	0.07	−0.28	**−0.51**
NO_3	−0.22	0.03	**−0.26**

lake in the database using the values of the predictor variables and the discriminant functions and then computing the number of correct group assignments. The reclassification exercise identified the correct group membership in 64% of the cases (Table 7). The class with the greatest success rate was Extinct.

Examination of these results suggests that two "superclasses" of fish status could be defined that would be readily predictable from lake chemistry. These larger groups are indicated by the boxes in Table 7 and might be called "Affected" and "Unaffected" populations of trout. The prediction success based on these larger groups is more than 89%.

Regression

As discussed above, using categorical variables as dependent variables in regression analyses is not strictly correct from a statistical point of view. However, previous studies have characterized the relationships between lake chemistry and fish status in Norway with regression models using fish status categories as dependent variables (e.g. Wright and Snekvik 1978; Muniz and Walløe 1990). To facilitate comparisons of our results with these earlier works, regression analyses were also performed in this study. We began with simple regression analyses using single chemical variables as the independent variable. The simple linear regressions (Table 8) using most of the chemical variables explained a statistically significant fraction of the variance in the trout response variables. pH and labile aluminum had the largest *r*-squared values for simple regressions, explaining 52–64% of the variance in the trout status variables. Total sulfate explained only 5% of the variance in single-variable regressions, and calcium only 12–17%. Thus, neither is a good predictor as an individual variable of trout response, for reasons discussed under principal component 1.

We also calculated Spearman's rank correlation coefficient (rho) for each of the trout response variables with each of the chemical variables used in simple regressions (Table 8). Like *r* in regression, rho ranges from −1.0 to +1.0. The same patterns emerge from this nonparametric analysis as for the previous analyses: the strongest correlations between the trout response variables and chemical variables are with Al_i, pH, and

TABLE 7. Results of reclassification of lakes using discriminant functions. Discriminant group 1 comprises all lakes with Healthy trout populations, including both Improved and Unchanged populations (see Tables 2 and 4). Group 2 comprises lakes with Marginal populations that have improved or remained Unchanged. Group 3 includes only lakes with Marginal populations that have declined during the period of record. Group 4 comprises only lakes that have lost their trout populations (Extinct) during the period of record. Overall, 64% of cases were correctly classified into four classes. Prediction success of two "superclasses" (boxed) was 89%.

Actual group	Group	*n*	Predicted group membership (%) 1	2	3	4
Healthy (Improved + Unchanged)	1	190	113 (60)	50 (26)	27 (14)	0 (0)
Marginal (Improved + Unchanged)	2	68	25 (37)	31 (46)	12 (17)	0 (0)
Marginal (Declined)	3	98	7 (7)	11 (11)	52 (53)	28 (29)
Extinct	4	228	1 (1)	4 (2)	44 (19)	179 (79)

TABLE 8. Regression analysis (N = 584 lakes). Simple regression coefficients (r), r-squared values, and Spearman's rank correlations (rho) for Trout Status (Status) and Change in Trout Status (Δ Status) with respect to chemical variables. All regressions and rank correlations with pH through sulfate were highly significant; other significant relationships are indicated individually. ns, not significant; *significant ($p < 0.05$); **significant ($p < 0.01$); ***all relationships with this variable or individual test significant ($p < 0.0001$).

Chemistry variables	Status r	Status r-squared	Status rho	Δ Status r	Δ Status r-squared	Δ Status rho
pH***	−0.74	0.55	−0.76	−0.80	0.640	−0.81
Al_i***	0.72	0.52	0.76	0.75	0.560	0.80
ANC***	−0.64	0.41	−0.71	−0.71	0.510	−0.77
Ca***	−0.35	0.12	−0.40	−0.41	0.170	−0.44
NO_3***	0.29	0.09	0.42	0.34	0.110	0.47
N-M SO_4***	0.26	0.07	0.31	0.25	0.060	0.30
SO_4***	0.22	0.05	0.32	0.23	0.050	−0.33
K	−0.19***	0.04***	−0.16**	−0.16***	0.02***	−0.15**
Mg	−0.14***	0.02***	(−0.12 ns)	−0.12***	0.01**	−0.11**
Na	−0.08*	0.01*	(−0.01 ns)	(−0.03 ns)	(0.00 ns)	(0.04 ns)
Cl	(−0.06 ns)	(0.00ns)	(0.05 ns)	(0.00 ns)	(0.00 ns)	0.11**
TOC	(0.00 ns)	(0.00ns)	(−0.00ns)	(−0.02 ns)	(0.00 ns)	(0.03 ns)

ANC. There are moderate associations between trout status and calcium, nitrate, nonmarine sulfate, and sulfate, and other correlations are weak.

We also performed multiple regression analyses using four different variable sets (Table 9). The first variable set ("physiological variables") consists of pH and Al_i, which have direct toxic effects, and calcium, which acts directly to ameliorate these effects. The second variable set comprises the seven main geochemical variables (Ca, Mg, Na, K, Cl, SO_4, and NO_3); the interactions among these ions control ANC, pH, and Al_i concentrations. The third variable set comprises the seven major ions (as in set 7) plus pH; these eight variables are usually available from survey information. The fourth variable set comprises all the independently measured chemical variables available from the survey data (no calculated variables were included in this set).

The results of the multiple regressions (Table 9) indicated that variable sets including pH and aluminum are the best predictors of trout status. Stepwise multiple regressions yield modest improvements (5–15%) over pH and aluminum as single predictors. Multiple regression using a combination of pH and the seven major ions have nearly the same predictive capacity as regressions based on pH and aluminum.

These results suggest that data sets lacking aluminum information may still be useful in characterizing the relationship between chemistry and fish status in nature, at least in situations similar to those in Norway. This is supported statistically through regression analysis (Table 9) and the strong intercorrelations among pH, aluminum, and alkalinity in the PCA results (Table 2) and physically through the pH-dependent dissolution and toxicological effects of aluminum. This in no way diminishes the importance of further studies on the complex chemistry and toxicological effects of aluminum; it merely relates to the interpretation of empirical relationships from survey information.

General Discussion

The extinction of local fish populations in Norway has occurred on a massive scale, and there is little doubt that acidic

TABLE 9. Regression analysis. *R*-squared comparisons for stepwise multiple regressions using Trout Status (Status) or Change in Trout Status (Δ Status) with four sets of variables. All regressions were highly significant ($p < 0.0001$).

	Dependent variable	
Stepwise regression	Status	Δ Status
Physiological variables		
Step 1: pH	0.550	0.639
Step 2: Al_i	0.593	0.669
Not included: Ca		
Major ions		
Step 1: Ca	0.122	0.170
Step 2: SO_4	0.364	0.456
Step 3: Mg	0.468	0.537
Step 4: K, NO_3	K: 0.480	NO_3: 0.549
Step 5: NO_3, K	NO_3: 0.496	K: 0.561
Not included: Na, Cl		
Major ions + pH		
Step 1: pH	0.550	0.639
Step 2: Cl	0.574	0.650
Step 3: SO_4	0.584	0.654
Step 4: K, Mg	K: 0.592	Mg: 0.662
Step 5: Mg, Cl removed	Mg: 0.595	(−Cl): 0.662
Step 6: Cl removed, —	(−Cl): 0.595	
Not included: Ca, NO_3, Na, Cl (Status); Ca, NO_3, K, Na, Cl (Δ Status)		
All measured variables		
Step 1: pH	0.550	0.639
Step 2: Cl	0.574	0.650
Step 3: Al_o	0.598	0.663
Step 4: Al_i	0.626	0.689
Step 5: Ca, Na	Ca: 0.631	Na: 0.692
Step 6: NO_3, —	NO_3: 0.639	
Step 7: Na, —	Na: 0.642	
Not included: SO_4, K, Mg, TOC (Status); Ca, NO_3, SO_4, K, Mg, TOC (Δ Status)		

deposition is the cause (Wright and Snekvik 1978; Rosseland et al. 1980; Sevaldrud and Skogheim 1986; Reuss et al. 1987; Henriksen et al. 1989). The scale of the problem in Norway can be illustrated with a comparison with an area severely affected by acidic deposition in the United States. Of 858 lakes with historical information on fish populations in the Adirondack region of New York State, 140 lakes, or 16%, have lost one or more fish populations apparently as a result of acidification (Adirondack Lakes Survey: Baker et al. 1990). In contrast, in a study of 891 Norwegian lakes with historical information on fish populations, 279 lakes, or 31%, have lost one or more fish populations (Thousand Lake Survey: Henriksen et al. 1988). Trout populations especially have been severely affected in Norway: of the 584 trout populations considered in this study, 228 (39%) have become extinct in recent decades, and 98 (17%) are in decline; thus, a majority (56%) of the trout populations in the survey have been adversely affected by acidic deposition.

Comparison of These Results with Recent Fish and Chemistry Surveys in Norway

Wright and Snekvik (1978) examined the relationships between lake chemistry and an index of fish community status (3: overpopulated; 2: good; 1: sparse; 0: barren) which did not distinguish among species, although the dominant fish species in their lakes were trout or perch. Their study of 700 lakes in four southernmost counties of Norway found no relationship between community status and sea salt concentrations. The present study supports their findings, with respect specifically to trout status. Wright and Snekvik found no relationship between aluminum and community status, but Sevaldrud and Skogheim (1986) correctly point out that only total aluminum concentrations were available to Wright and Snekvik. Much of the monomeric aluminum would occur as nontoxic Al_o in humic lakes. Following a survey of 623 lakes (including some of Wright and Snekvik's lakes), Sevaldrud and Skogheim suggested a strong qualitative relationship between community status and Al_i, the most toxic fraction of total aluminum. In stepwise multiple regressions using fish community status, calcium and pH emerged as the first and second variables in Wright and Snekvik's analysis; the importance of these variables was supported by Sevaldrud and Skogheim and is confirmed by the present study. The secondary importance of pH relative to calcium in Wright and Snekvik, which is reversed in the present study, may be partly explained by the difference in the dependent variables used. The present study uses the status of a single species whereas Wright and Snekvik's index includes effects on trout, perch, and other species. Many studies have confirmed that brown trout are less tolerant to low pH than perch; thus, a given pH might be associated with a damaged community in a trout lake, and a healthy community in a perch lake, making the relationship between *fish community status* and *pH* weaker than the relationship between specifically *trout status* and *pH* found in this study. Also, Wright and Snekvik's lakes had a wider range of calcium values, which might increase the relative predictive capacity of calcium in their report. Thus, differences in the findings of Wright and Snekvik versus the present study in the relative importance of calcium and pH appear to be understandable in view of the variables used and range of variables in the two data sets while confirming (together with Sevaldrud and Skogheim) the general importance of pH and calcium concentrations to fish populations.

In contrast with the present study and two previous works (Wright and Snekvik 1978; Sevaldrud and Skogheim 1986), Muniz and Walløe (1990) concluded that the concentration of calcium had no influence on the status of brown trout populations in Norway. This finding is important because it deals specifically with brown trout, and it is based on a large number of populations (including those in this study); however, it appears to rest on several flaws in interpretation. The conclusion that calcium has no influence on trout populations rests primarily on a stepwise linear regression ($N = 1192$) of trout status on two physiographic variables (lake area and elevation) and 12 chemical variables. pH and labile aluminum were the first and second variables to be entered in the stepwise regression and the only chemical variables which made a significant contribution to the regression. The third and last variable was lake elevation, resulting in a multiple *R*-squared of 0.44, lower than any multiple *R* in the present study. The absence of a significant calcium effect led the authors to conclude that it was unimportant for trout status. This appears to ignore the fact that the contribution an independent variable makes to a multiple regression is very much dependent on the other variables in the model. A more conservative conclusion is that, in the presence of information on pH, elevation, and labile aluminum, calcium provides no further predictive information. Muniz and Walløe mentioned other unpublished multi- and bivariate analyses

which also identified no calcium effect. Muniz and Walløe did not address the preeminent role of calcium in determining ANC and pH, and hence labile aluminum concentration, and therefore trout status. Calcium makes the largest single contribution to explained variance in ANC (39%) in the 584 lakes in the present study, and calcium plus sulfate account for 89% of the variance in ANC, based on a stepwise multiple regression using the seven major ions with ANC as the dependent variable. Muniz and Walløe also did not consider that the response of a trout population to calcium is, to a certain extent, sulfate dependent as discussed in this article.

Consideration of Remaining Uncertainties

The relationships among chemistry variables and the trout response variables are robust (Tables 3 and 4), and the prediction successes from the discriminant model are quite good for biological systems. Nevertheless, it is reasonable to ask why the relationships are not even stronger, considering the direct toxic effects of some of the chemical variables. The answer may be due in part to time lags in the response of trout populations to acidification, and also in part due to the quantal nature of the response variables. The first sign of damage to trout populations in acidifying watersheds is usually recruitment failure, probably linked to acidic episodes in the spring when sensitive trout fry emerge from gravel stream beds (Leivestad et al. 1976; Rosseland et al. 1980; Gunn and Keller 1984; Reader and Dempsey 1989). The adult trout population under these circumstances may persist for years, especially if the lake as a whole is less influenced by acidic events than its headwater streams. Eventually the population consists of only a few older year classes. During this time, the water chemistry of the lake itself gradually deteriorates. There are thus a range of chemistries associated with only a single trout status category: Marginal in the case of present Trout Status, or Declining in the case of Change in Trout Status. Furthermore, the category Marginal includes lakes with good water quality which have always had sparse populations for reasons other than acidification. Due to continued acidic loading, water quality can also continue to deteriorate in Extinct lakes long after they can support trout. This range of water chemistry values is also only associated with a single trout status category Extinct. Thus, time lags in population response, together with the integer values of the trout status variables, reduce to some extent the predictability of trout status.

Conclusions

Trout status in Norway is strongly related to pH, the concentration of toxic aluminum, and alkalinity (calculated from the seven major ions). Calcium and sulfate influence trout status moderately as individual variables, but quite strongly in combination, since the difference in their concentrations is the major determinant of alkalinity in these lakes. Since most of the sulfate in these lakes derives from atmospheric inputs (Henriksen et al. 1988), trout status is strongly influenced by acidic deposition. The causal chain includes the deposition of sulfate and its effects on alkalinity and pH, and the resulting mobilization of toxic aluminum; thus the effects can be traced from atmospheric transport to membrane dysfunction.

The discriminant and regression models developed here provide the basis to compare predictions of trout status from different kinds of chemical information. pH, labile aluminum, and ANC are the best single predictors; variable sets including both pH and labile aluminum are the best predictors in multiple regressions. However, a variable set including pH and the seven majors ions, which are more commonly available, especially in older data sets, was also a good predictor of trout population response. This finding demonstrates the continued usefulness of older data sets lacking aluminum information in the study of fish response to water chemistry. This is supported by the strong correlations among pH, ANC, and labile aluminum. These empirical models relating lake chemistry and fish population response may be used in future work to estimate the effects on trout populations following changes in atmospheric deposition and lake water chemistry.

Acknowledgements

We are grateful to R. F. Wright, B. O. Rosseland, S. C. Tremaine, and S. A. Norton for valuable discussions throughout the course of this work. R. F. Wright, S. C. Tremaine, C. K. Minns, George Hornberger, and an anonymous reviewer read the manuscript and suggested many improvements. The work was supported in part by Senior Scientist Research Fellowships from the Royal Norwegian Council for Scientific and Industrial Research (NTNF) to Bulger and Cosby and in part by the Norwegian Institute for Water Research (NIVA).

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Suspended Sediment Particles Inside Gills and Spleens of Juvenile Pacific Salmon (*Oncorhynchus* spp.)

Dennis W. Martens and James A. Servizi

Department of Fisheries and Oceans, Cultus Lake Salmon Research Laboratory, Cultus Lake, B.C. V0X 1H0, Canada

Martens, D. W., and J. A. Servizi. 1993. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). Can. J. Fish. Aquat. Sci. 50: 586–590.

Intracellular sediment particles were observed in the gills of underyearling coho salmon (*Oncorhynchus kisutch*) and pink salmon (*O. gorbuscha*) following laboratory exposure to Fraser River sediment. Gills of underyearling sockeye salmon (*O. nerka*), chinook salmon (*O. tshawytscha*), and coho exposed to a natural suspended sediment in Cultus Lake hatchery water also contained intracellular mineral particles. Mineral particles were seen in both epithelial and underlying gill filamental cells, and it is believed that these particles were phagocytosed by the former. Intracellular sediment particles were also observed in spleens of some sediment-exposed fish. Electron microscopy was used to measure gill particle sizes and X-ray diffraction analysis to identify eight minerals and one metal in the gills of sockeye previously exposed to suspended sediment.

Chez le saumon coho (*Oncorhynchus kisutch*) et le saumon rose (*O. gorbuscha*), on a observé dans les branchies d'alevins de la grosseur du doigt des particules d'origine sédimentaire qui s'étaient logées dans des cellules après exposition de sujets au laboratoire à des sédiments du Fraser. Les branchies d'alevins de la grosseur du doigt chez le saumon rouge (*O. nerka*), le saumon quinnat (*O. tshawytscha*) et le saumon coho exposés à une suspension naturelle de sédiments dans de l'eau de la pisciculture de Cultus Lake, contenaient aussi des particules minérales intracellulaires. Ces particules étaient trouvées dans les cellules épithéliales ainsi que dans les cellules des filaments branchiaux sous-jacents; on pense que ces particules ont été phagocytées par les cellules épithéliales. On a aussi observé de ces particules dans les cellules de la rate de certains poissons exposés à des sédiments. La granulométrie de ces particules a été déterminée par microscopie électronique, et l'analyse par diffraction des rayons-X a permis d'identifier huit minéraux et un métal dans les branchies de saumons rouges précédemment exposées à des sédiments en suspension.

Received December 3, 1990
Accepted September 9, 1992
(JA831)

Reçu le 3 décembre 1990
Accepté le 9 septembre 1992

Periodic exposure to suspended solids (SS) is an integral part of freshwater life history for many salmonids. Most populations are exposed to high concentrations of natural SS during heavy rainfall or spring freshets, but habitat alterations such as placer and gravel mining or navigational channel dredging may also increase SS significantly in the aquatic environment. The direct effects of SS exposure to fish over a wide range of concentrations have been the subject of numerous investigations in the past. Observed effects include mortality (Servizi and Martens 1987, 1991), avoidance, cough response, and elevated plasma glucose (Servizi and Martens 1992), depressed leucocrit (McLeay et al. 1987), and reduced disease resistance (Redding et al. 1987). Owing to direct contact with SS, gills are most affected and several, but not all, studies report significant histopathological alterations in gill fine structure following exposure to sublethal as well as lethal SS concentrations. Documented branchial histopathologies include hypertrophy and fusion of lamellae (Herbert and Merkens 1961; Southgate 1962) and necrosis (Servizi and Martens 1987). In addition to histopathological changes in gill structure, intracellular incorporation of mineral particles by branchial phagocytosis has also been documented in fish following SS exposure (Goldes et al. 1986).

The objectives of this study were to identify and measure intracellular sediment particles in the gills of four species of Pacific salmon (*Oncorhynchus* spp.) and in the spleens of three of them and to determine whether there is a relationship between intracellular particle frequency and SS exposure concentrations.

Materials and Methods

Fish and Sediment

Fish used for study of intracellular sediment particles included coho salmon (*Oncorhynchus kisutch*) and pink salmon (*O. gorbuscha*) from Chilliwack River stocks, sockeye salmon (*O. nerka*) from the Upper Pitt River stock, and chinook salmon (*O. tshawytscha*) from the Quesnel River stock. Fish were obtained as eggs and incubated and reared in Cultus Lake water (Servizi and Martens 1991) at Cultus Lake Laboratory. Coho, sockeye, and chinook salmon were fed Oregon Moist Pellet and pink salmon were fed Biodiet. All fish were starved 24 h prior to sediment bioassay or sampling directly from the hatchery water (HW).

Groups I and II (Table 1) were used in 96-h (acute) bioassays where test specimens were exposed to relatively high concentrations of suspended Fraser River sediment according to procedures previously described (Servizi and Martens 1987). Group IIIa sockeye were exposed to sediment in HW as described below, followed by 96 h of exposure to 5.0 g Fraser

TABLE 1. Details of fish exposures and histological examinations.

Fish exposures				Histological examination[a]		
Group	Species	Size (g)	SS exposure	Organ	No. of fish	EM, LM, X
I	Coho	0.26–0.45	0–41 g·L^{-1} (96 h)[b]	Gill	32	LM
II	Pink	0.23–0.25	0–21 g·L^{-1} (96 h)[b]	Gill	24	LM
IIIa	Sockeye	5.50–6.50	HW[c] and 5 g·L^{-1} (96 h)[b]	Gill	4	EM, X
IIIb	Sockeye	5.50–6.50	HW[c]	Gill	3	LM, EM, X
				Spleen	3	LM
IV	Coho	3.90–5.20	HW[c]	Gill	3	LM
				Spleen	3	LM
V	Chinook	4.40–6.80	HW[c]	Gill	3	LM
				Spleen	3	LM
VI	Sockeye	5.50–6.50	HW[c]	Gill	2	LM

[a]EM, electron microscopy; LM, light microscopy; X, X-ray diffraction.
[b]Fraser River sediment exposure.
[c]HW (hatchery water); 200 mg SS·L^{-1} (26 November 1986) decreasing to 61 mg·L^{-1} (13 December 1986), 4 mg·L^{-1} (8 January 1987), and 0 mg·L^{-1} (5 February 1987).

River sediment·L^{-1}. Fraser River sediments used in the bioassays had more than 90% of particles less than 5 μm in geometric mean diameter (Servizi and Martens 1991).

Groups IIIb, IV, V, and VI were examined for intracellular sediment particles following chronic exposure to relatively lower SS concentration in the HW supply (Table 1). Owing to a rainstorm, SS in HW increased to 200 mg·L^{-1} on 26 November 1986 from a normal value less than 0.2 mg·L^{-1}. By 13 December, SS had declined to 61 mg·L^{-1}, then to 4 mg·L^{-1} (8 January 1987), and finally to 0.2 mg·L^{-1} (5 February).

Histological Sampling and Examination

Fish were killed by 2-phenoxyethanol overdose and the first and second gill arches of sockeye, coho, and chinook underyearlings were excised and placed immediately in histological fixative. Pink salmon fry were placed whole into fixative. In the case of sockeye underyearlings, where both light microscopic (LM) and electron microscopic (EM) examinations were scheduled, gill arches one and two were taken from both sides, one side for LM and the other for EM. Spleens from the first three species (not pink salmon) were carefully excised and rinsed in distilled water before being placed in fixative separate from the gills. This procedure was used to avoid surface contamination of the spleen by sediment particles from the gill.

Sockeye gill samples scheduled for EM examination were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer at 4°C for 24 h prior to transfer to sodium cacodylate buffer for storage at 4°C. Further processing of gill samples for examination included distilled water surface rinses, 30-min staining in 2% uranyl acetate, and dehydration in absolute alcohol followed by two changes in propylene oxide. Finally, gill samples were embedded overnight at 70°C in epoxy resin (Spurr). Sections were cut at 0.25 μm with a Reichert Ultracut microtome. Examinations and particle size measurements (longest axis) were made with a Phillips 400 transmission EM and the mineral identity of observed sediment particles was determined by energy dispersive X-ray spectroscopy (Kevex Corp.). Gill examinations by EM were designed to provide information on the presence or absence of intracellular particles and to identify and measure observed sediment particles, but particle frequency was not determined by EM. All EM sample preparations and examinations were conducted at the Department of Pathology, School of Medicine, University of British Columbia, Vancouver, B.C., Canada.

Specimens scheduled for LM examination were fixed in Bouin's solution for 24 h followed by transfer to 70% ethanol for storage. Specimens were later prepared for examination by paraffin embedding, sectioning at 3–5 μm, and staining with hematoxylin and eosin. Slides were coded so that initial examinations were "blind."

Particle frequencies in the gills were determined by LM counting of all intracellular particles in each lamellae examined and in the adjacent filament area. In each fish examined, counts were made for 50–150 randomly selected lamellae from several different filaments. Particle frequency was expressed as particles per 100 lamellae examined. ANOVA (GLM) and Student's t were used for statistical comparisons.

Results

Particles in Gills: EM and X-Ray Diffraction

Intracellular mineral particles appeared irregular and angular in shape and were electron dense (dark) under EM (Fig. 1). Many sediment particles were present in epithelial cells and were frequently found within membrane-bounded structures (vacuoles). Epithelial cells were identified by pronounced ridging and desmosomal attachments to adjacent cells (Fig. 1). Particle identities were determined by X-ray diffraction, followed by particle size measurement which caused fragmentation of the target particle (Fig. 1). No cytopathological changes were evident.

A total of 182 sediment particles were detected and measured by EM of seven sockeye gill samples (Table 2). Mean sizes (based on longest axis) of sediment particles ranged from 0.27 to 0.54 μm, while the overall range was 0.10 to 2.5 μm. Mean particle sizes were similar for both groups of fish. Eight minerals and one metal (titanium) were detected by X-ray diffraction. Biotite, which included chlorite and vermiculites, accounted for 49.5% of the 182 sediment particles followed by mica at 30%. Feldspar and chrysotile accounted for 7 and 5%, respectively. Kaolin, silica, talc, sphene, and titanium were

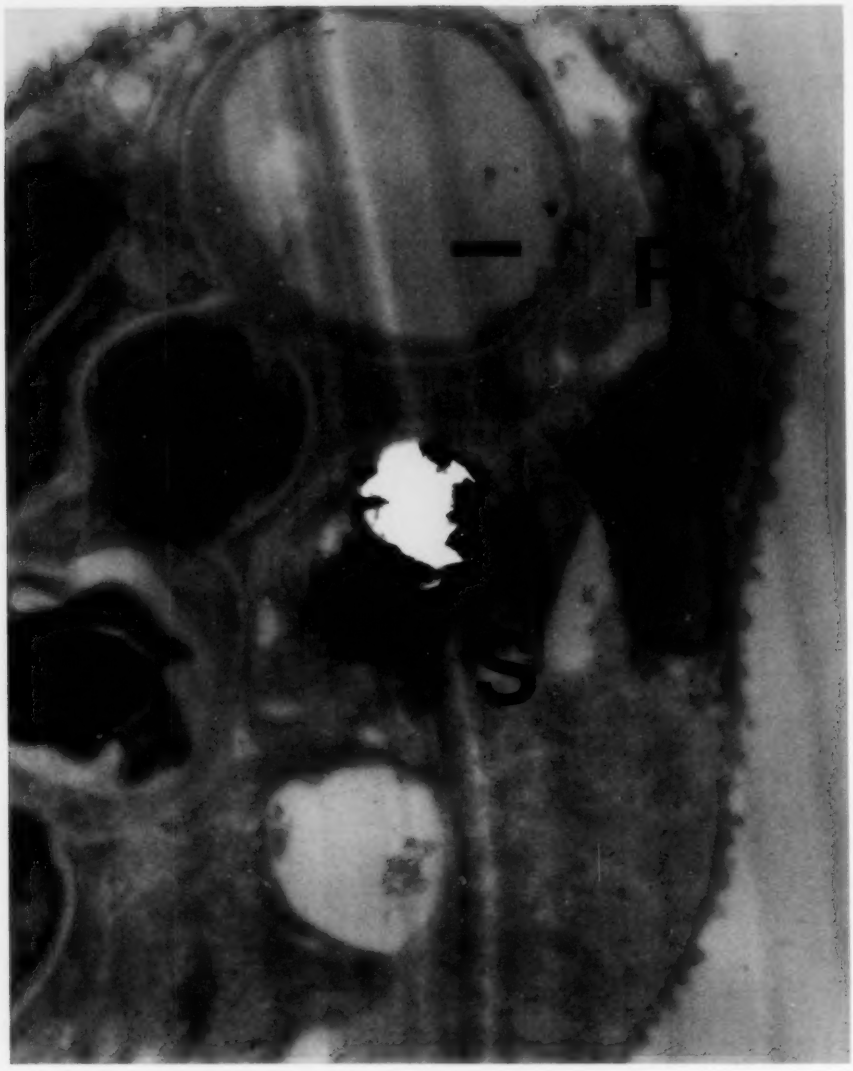

FIG. 1. Sediment particle (S) within Group IIIb sockeye lamellar epithelium, R, ridging on epithelial cell surface. A portion of the particles was fragmented by size measurement. Bar = 0.25 μm.

TABLE 2. Particle sizes and numbers of specific mineral particles identified in seven sockeye gill samples (Groups IIIa and IIIb). Sizes determined by electron microscopy and minerals by X-ray diffraction.

Group	*N*	Particle size (mean ± SD)[a]	Number of particles of each mineral[b]								
			Bi	Mi	Fel	Ch	Kao	Si	Ta	Sp	Ti
IIIa	31	0.32 ± 0.29	25	4	0	1	0	1	0	0	0
	11	0.32 ± 0.09	1	9	1	0	0	0	0	0	0
	29	0.27 ± 0.12	19	4	3	0	3	0	0	0	0
	29	0.36 ± 0.18	3	22	0	2	0	1	0	1	0
IIIb	32	0.33 ± 0.18	16	4	2	3	1	1	3	1	1
	29	0.39 ± 0.21	19	7	2	1	0	0	0	0	0
	21	0.54 ± 0.36	7	5	5	2	1	1	0	0	0

[a]Longest axis.

[b]Bi, biotite (includes chlorite and vermiculite); Mi, mica; Fel, feldspar; Ch, chrysotile; Kao, kaolin; Si, silica; Ta, talc; Sp, sphene; Ti, titanium (metal).

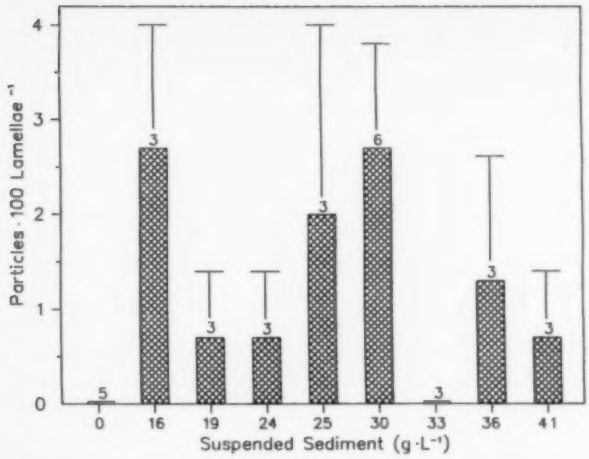

FIG. 2. Mean frequency and standard error of gill intracellular sediment particles for coho salmon (Group I) exposed to Fraser River SS for 96 h. Mean frequency is 1.5 particles·100 lamellae^{-1} (SE = ±0.4) over the range 16–41 g SS·L^{-1}. Numbers at the tops of columns are the fish examined.

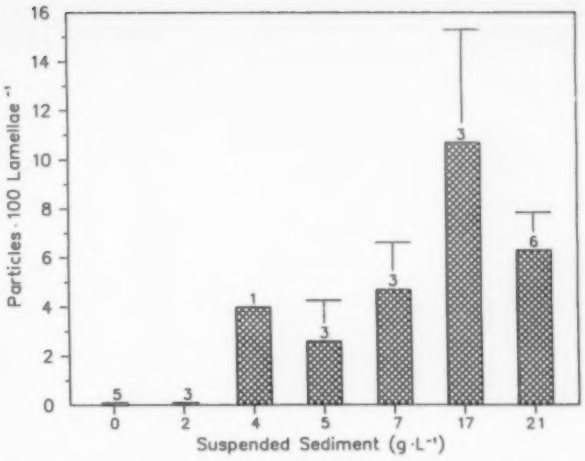

FIG. 3. Mean frequency and standard error of gill intracellular sediment particles for pink salmon (Group II) (0.23–0.25 g) exposed to Fraser River SS for 96 h. Mean frequency is 6.0 particles·100 lamellae^{-1} (SE = ±1.1) over the range 4–21 g SS·L^{-1}. Numbers at the tops of columns are the fish examined.

minor constituents and collectively made up the remaining 8.5%. Except for talc and titanium which were minor constituents observed only in Group IIIb, the gill particle mineralogy was similar for both groups.

Particles in Gills and Spleens: LM Examinations

Intracellular sediment particles in tissues, including gills, as observed by LM, were irregular and angular, yellow to gold in colour, and exhibited varying degrees of light refractivity. Intracellular particles of HW origin were similar in size and appearance to those of Fraser River sediment origin. Particles were observed in the epithelial cells of the gill lamellae but were also frequently observed deep within the parenchyma of the gill filament. Many of the particles observed in the parenchyma were several cells inside the epithelial layer. No cytopathological changes were observed in the tissues examined.

Particle frequencies in coho fry gills ranged from 0 to 2.7·100 lamellae^{-1} following 96-h exposures to sediment concentrations ranging from 16 to 41 g·L^{-1} (Fig. 2). Mean particle frequency was 1.5 ± 2.1·100 lamellae^{-1} over the exposure range 16–41 g SS·L^{-1}. Particle frequency was not correlated with SS exposure concentration ($r = 0.15$, $p > 0.05$). Particles were not seen in the 250 lamellae examined from five control fish taken from sediment-free water.

Pink salmon fry gills contained intracellular particles following 96 h of exposure to 4.3 g SS·L^{-1} and greater, but particles were not observed when fish were exposed to 1.6 g SS·L^{-1} or to sediment-free water (Fig. 3). Intracellular particle frequency was a function of SS exposure concentration ($r^2 = 0.666$) and the linear correlation was significant ($p < 0.05$).

Gills of pink salmon fry averaged 6.0 particles·100 lamellae^{-1} (SD = ±4.5) for SS exposures over the range 4–21 g SS·L^{-1}. Coho gills averaged only 1.5 ± 2.1 particles·100 lamellae^{-1} for an exposure range of 16–41 g SS·L^{-1}. The difference in mean particle frequencies between pink and coho salmon fry gills was highly significant ($p < 0.001$) and suggests that branchial phagocytic activity toward SS may be species related.

TABLE 3. Intracellular mineral particles in salmon gills and spleens on 8 January 1987 (Groups IIIb, IV, and V) and 9 February 1987 (Group VI), following a rise of SS to 200 mg·L^{-1} in hatchery water supply on 26 November 1986. Means with the same superscript are not significantly different.

Group	Species	*N*	Particles·100 lamellae^{-1} (mean ± SD)	Particle presence in spleen tissue	
				Presence	Absence
IIIb	Sockeye	3	14.6^a ± 2.5	3	0
IV	Coho	3	2.4^b ± 1.0	1	2
V	Chinook	3	0.9^b ± 1.6	1	2
VI	Sockeye	2	9.1^a ± 4.3	Not examined	

Intracellular mineral particles were present in gills and spleens of sockeye, coho, and chinook when sampled from hatchery troughs on 8 January 1987 (Table 3). For sockeye gills the mean particle content was 14.6 ± 2.5·100 lamellae^{-1} which was signfiicantly higher ($p < 0.01$) than the mean particle contents for coho (2.4 ± 1.0) and chinook (0.9 ± 1.6). The difference between coho and chinook lamellae mean particle frequencies was not significant ($p > 0.05$). The mean lamellae particle frequency for sockeye decreased to 9.1 ± 4.3 when they were sampled on 9 February, but the difference was not significant ($p > 0.05$) when compared with the sockeye sampled a month earlier. The spleens of all three sockeye sampled 8 January contained SS particles whereas particles were seen in spleens of only one of three coho and chinook (Table 3).

Discussion

We believe this to be the first report to quantify the frequency of mineral particles in gill tissues. However, evidence for a relationship between intracellular particle frequency and SS exposure level ranged from nil (coho) to weak (pink). These results suggest that there is little likelihood of determining SS exposure history from gill intracellular particle frequency. The

average frequency of intracellular mineral particles ranged from zero for pink and coho sampled from sediment-free water up to 14.6·100 lamellae^{-1} for sockeye chronically exposed to low levels of SS. There was also evidence of species differences in branchial phagocytic activity, with pink fry surpassing coho during acute exposure to high levels of SS. In addition, sockeye phagocytic activity exceeded both coho and chinook during chronic exposure to relatively lower SS. Duration of exposure to SS appeared to play a role in phagocytosis, since there was no difference in intracellular particle frequencies for coho exposed acutely to high SS and those exposed chronically to relatively low SS. However, size or age may also be a factor, since chronically exposed Group IV coho were larger and older than acutely exposed Group I coho.

There may be a physiological basis for a weak or nonexistent relationship between intracellular particle frequencies and acute SS exposure levels. SS exposure has been shown to induce stress in juvenile coho (Servizi and Martens 1992), and the stress associated with SS exposure may act physiologically to suppress phagocytosis. Cortisol (Stave and Robertson 1985) and stress or cortisol (MacArthur and Fletcher 1985) have been shown to affect activity of internal fish phagocytes. Assuming similar behaviour of epithelial and internal phagocytes, the stress of SS exposure may have suppressed phagocytosis of mineral particles in the gills and influenced particle frequencies.

The long dimensions of intracellular mineral particles averaged about 0.5 μm or less, which makes these particles less than one-tenth the diameter of epithelial cells. The mineral particles phagocytosed were representative of the smallest particles which occur in natural sediments in the area (Servizi and Martens 1987, 1991). Evidently the larger sediment particles were not phagocytosed even though they were in contact with gill tissues. The foregoing suggests a possible particle size limitation for phagocytosis by epithelial cells.

The occurrence of mineral particles deep in the parenchyma and in splenic tissues suggests that the particles were transferred to those locations from epithelial phagocytes. Movement of mineral particles to the internal compartments of the fish requires that the intracellular particles first be released from epithelial phagocytes. There is evidence to suggest that such releases may occur. For example, George et al. (1976) observed phagocytosis of mineral particles from the aquatic environment by epithelial cells of mollusc gills, and the particles were later exocytosed and finally endocytosed in the gills by amoebocytes. A similar process in salmon would help to explain the presence of intracellular mineral particles within the gill parenchyma and the spleen.

Kaolin, one of the minor mineral particles identified in sockeye gills, is cytotoxic to mammalian macrophages (Davies 1983) but its cytotoxicity to fish phagocytic cells is not known. In our study, kaolin was detected in two sockeye gill samples (Table 2) after chronic exposure but no cytopathological changes were observed. This result corroborates an earlier observation by Goldes et al. (1986) concerning phagocytosis of kaolin particles during acute exposure. However, some toxic metals and organic chemicals are often bound to suspended particulates and bottom sediments (Allen 1986). Thus, phagocytic uptake of contaminated particles may provide an entry pathway for toxic substances.

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Nutritional Condition of the Pacific Lamprey (*Lampetra tridentata*) Deprived of Food for Periods of Up to Two Years

John N. C. Whyte, Richard J. Beamish, Norma G. Ginther, and Chrys-Ellen Neville

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Whyte, J. N. C., R. J. Beamish, N. G. Ginther, and C.-E. Neville. 1993. Nutritional condition of the Pacific lamprey (*Lampetra tridentata*) deprived of food for periods of up to two years. Can. J. Fish. Aquat. Sci. 50: 591–599.

The anadromous parasitic Pacific lamprey (*Lampetra tridentata*) does not feed during metamorphosis or its spawning migration. To assess the utilization of body reserves, we compared the compositions of an adult lamprey held for 2 yr without food, recently metamorphosed lampreys, and lampreys starved for 6 mo. Moisture was higher and soluble ash and lipid levels were lower in tissue of the 2-yr-starved than in metamorphosed lampreys (2.67 and 3.39 $kJ \cdot g^{-1}$, respectively). Fatty acid profiles of 2-yr-starved and metamorphosed lampreys were qualitatively similar except for the presence of 15:0 in the latter. Substantially lower levels of 14:0 and 16:1*n*7 and higher levels of polyunsaturated fatty acids occurred in starved lampreys. Changes in composition of lampreys starved for 6 mo were similar to changes between the 2-yr-starved and metamorphosed lampreys. Maintenance energy in a normalized 1-g lamprey starved for 6 mo was derived from catabolism of 71% lipid and 29% protein; total loss was 2.56 kJ or 49% of the original energy. The ability to survive extended periods on endogenous reserves, which may have allowed *L. tridentata* to survive past environmental catastrophes, allows it to migrate considerable distances to spawning areas in the headwaters of rivers.

La lamproie du Pacifique parasite anadrome (*Lampetra tridentata*) ne s'alimente pas durant sa métamorphose ni durant sa migration de frai. Pour évaluer ses réserves corporelles, nous avons comparé la constitution de lamproies adultes privées de nourriture pendant 2 ans, des lamproies récemment métamorphosées et des lamproies privées de nourriture pendant 6 mo. La teneur en eau était plus élevée et la concentration de cendres solubles et de lipide étaient plus faibles dans les tissus des lamproies privées pendant 2 ans que dans ceux des lamproies récemment métamorphosées (respectivement 2,67 et 3,39 $kJ \cdot g^{-1}$). Les profils des acides gras chez les lamproies privées pendant 2 ans et les lamproies récemment métamorphosées étaient semblables du point de vue qualitatif, sauf pour ce qui est de la présence d'acides 15:0 chez ces dernières; chez les lamproies privées d'aliments, on a observé des concentrations d'acide 14:0 et 16:1*n*7 substantiellement plus faibles et des concentrations d'acides gras polyinsaturés plus élevées. Les modifications dans la constitution des lamproies privées d'aliments pendant 6 mo étaient semblables aux modifications que l'on observe entre les lamproies privées pendant 2 ans et les lamproies métamorphosées. L'énergie d'entretien chez une lamproie normalisée de 1 g privée pendant 6 mo a été obtenue à partir du catabolisme lipidique (71 %) et protéique (29 %); les pertes totales ont été de 2,56 kJ ou 49 % de l'énergie initiale. La capacité de survivre pendant longtemps sur des réserves endogènes, qui peut avoir aidé *L. tridentata* à survivre à des catastrophes environnementales dans le passé, lui permet d'entreprendre de longues migrations pour aller frayer dans les eaux d'amont.

Received April 9, 1992
Accepted August 26, 1992
(JB465)

Reçu le 9 avril 1992
Accepté le 26 août 1992

The Pacific lamprey (*Lampetra tridentata*) is a common anadromous fish, ranging from California to the Aleutian Islands, along the eastern coast of Russia and south to Japan. Larvae of the Pacific lamprey live up to 7 yr before metamorphosing into young adults (Russell 1986; Beamish and Northcote 1989; Beamish and Levings 1991). Larval lamprey live in burrows in the silt deposits of rivers, where they filter-feed on algae, detritus, bacteria, and protozoa (Moore and Mallatt 1980). The energy reserves accumulated during this larval stage are used during metamorphosis, the nontrophic period, when the characteristic features of the adult are developed. All parasitic and nonparasitic species of lamprey stop feeding during metamorphosis. However, the nonparasitic species no longer feed and spawn approximately 10 mo after metamorphosis.

Most larval Pacific lamprey metamorphose at age 4–5 yr. After metamorphosis the young adults begin to migrate into the salt water over an 11-mo period starting in September (Beamish and Northcote 1989; Beamish and Levings 1991). In the Nicola River in British Columbia, over 90% of the young adults migrate out of the river in early spring (Beamish and Levings 1991). Young adults feed parasitically on saltwater fish for a period generally considered to be about 1 yr (Beamish and Levings 1991). However, the trophic phase of development in salt water may differ among populations, as mature Pacific lamprey range widely in size and some have continued to feed in salt water in the laboratory for 4 yr (Beamish 1980). The return migration into fresh water can occur immediately before spawning or almost 1 yr prior to spawning, and although spawning occurs mainly in spring, it has been recorded as late as July (Beamish 1980).

When adult anadromous parasitic lamprey return to fresh water, they stop feeding and lose the ability to osmoregulate in salt water (Youson 1981). Cessation of feeding, or starvation,

is accompanied by rapid atrophy of the intestine, and any further assimilation of exogenous reserves, at least by intestinal absorption, is impossible (Youson 1981). The endogenous reserves accumulated during saltwater feeding are then used for upstream migration to the spawning area and for gonadal maturation and spawning. During the period from entry into fresh water until spawning occurs the Pacific lamprey shrinks about 20% in length (Beamish 1980). As Pacific lamprey do not feed on reentering fresh water or while being held in fresh water in the laboratory, the term "starved" used throughout the text expresses only the natural state of the fish and not any intentional deprivation of food. Nothing is known of the changes in body reserves of the Pacific lamprey during the period of prolonged starvation.

Changes in body reserves have been studied in the trophic and nontrophic stages of the anadromous parasitic sea lamprey (*Petromyzon marinus*) (Lowe et al. 1973; Beamish et al. 1979; Farmer 1980), river lamprey (*Lampetra fluviatilis*) (Moore and Potter 1976; Heikkala et al. 1984), and the Southern Hemisphere lamprey (*Geotria australis*) (Bird and Potter 1981, 1983a, 1983b). Anabolic and catabolic changes have also been investigated in the life cycle of the nonparasitic European brook lamprey (*Lampetra planeri*) (Moore and Potter 1976) and southern brook lamprey (*Ichthyomyzon gagei*) (Beamish and Legrow 1983). These studies indicated that the major source of maintenance energy during periods of food deprivation was derived from lipid, although some protein was also catabolized.

Pacific lamprey captured during upstream migration in June 12, 1986, survived in fresh water in the laboratory for 2 yr, the longest starvation period recorded for this fish. To assess the body reserves used during nontrophic stages of the Pacific lamprey, the biochemical composition, caloric content, and energy reserves of different tissues of the 2-yr-starved lamprey were compared with those from recently metamorphosed lampreys and with those from lampreys analyzed during a 6-mo holding, or starvation, period.

Materials and Methods

Lamprey Collection, Holding, and Processing for Analysis

Pacific lamprey (150) returning to the Somass River drainage system (Beamish and Northcote 1989) were captured at a fish bypass on June 12, 1986, and transported directly to the Pacific Biological Station, Nanaimo, British Columbia. They were kept in tanks containing well-aerated fresh water at 10 ± 2°C. The tank bottoms were covered with sand and rocks to provide cover and spawning areas. Lampreys were checked daily for mortalities and for the development of fungal disease. All but three males had either spawned or died by June 1987, and only two survived to the spring of 1988 when one was preserved. On May 10, 1988, the remaining lamprey was killed and the body dissected into head (that portion from mouth to just behind the last gill pouch), body (the remainder of the exterior body), liver, and remaining internal organs. There were insufficient gonads for separation and analysis. The body parts were freeze-dried, homogenized, vacuum packed, and stored at −80°C for subsequent analysis as parts of the "2-yr-starved" lamprey.

Recently metamorphosed Pacific lamprey were collected from the Fraser River on June 24, 1988. On return to the laboratory, two specimens were dissected into head, body (the remainder of the exterior body), liver, and remaining internal organs. Body parts were combined, freeze-dried, and homogenized for analysis as "metamorphosed" lamprey. Sufficient liver was available only for fatty acid analysis, and the gonads were insufficient in amount to be separately analyzed.

On October 27, 1988, a small sample of Pacific lampreys, which probably entered the Somass River in late July, was collected at Stamp Falls. Specimens (six) were held in tanks, as mentioned previously, and sampled for analysis after 0, 91, and 182 d of holding, and starvation. Lampreys collected were dissected into head, body (the remainder of the exterior body), liver, gonad, and remaining internal organs. The body parts were freeze-dried, homogenized, vacuum packed, and stored at −80°C until analyzed.

Biochemical Analysis

Analysis of chemical components in freeze-dried tissue has been described in detail previously (Whyte and Englar 1982; Whyte et al. 1986, 1987). Energy conversion factors used for lipid, carbohydrate, and protein were 35.24, 17.16, and 18.00 kJ·g dry weight^{-1}, respectively (Beukema and De Bruin 1979).

Methyl esters of fatty acid components of total lipids in freeze-dried lamprey parts were prepared by in situ saponification and methylation with methanolic boron trifluoride (Whyte 1988). Analyte solutions were separated on a Supelcowax 10 fused silica capillary column (30 m × 0.32 mm ID, 0.25-μm film) maintained at 190°C for 32 min and then programmed to 240°C at 2°C·min^{-1} and held at 240°C for 16 min. Helium carrier gas was controlled at 21 cm·s^{-1} with a split ratio of 100:1. Identification was made by comparison with standards and peaks assigned in accord with the data presented by Ackman (1986). Peaks of less than 0.2% of the total area were omitted from the profile. Fatty acid structure is represented as (L:BnX) where L = chain length, B = number of ethylenic bonds, and nX = position of the double bond closest to the terminal methyl group in monomer or *cis* methylene-interrupted structures. The expression Σ4-6,n3/poly. represents the ratio of the sum of higher unsaturated fatty acids (HUFA's) with four to six double bonds in the n3 series of acids to the sum of polyethylenic acids. Linear regression was performed on arcsine-transformed data (Zar 1984).

Results

The six lamprey (four males, two females) starved for 6 mo lost weight at a linear rate of 0.1628 g·d^{-1} (r^2 = 0.7571, p = 0.024) (Table 1). The head and body of the male lampreys formed a higher percentage of the whole body weight than that of the females (Table 1). This resulted from the higher content of gonads in the females. The gonadosomatic ratio of the males declined with starvation whereas that of the females increased (Table 1). Under starvation conditions the percentage of liver, the hepatosomatic ratio, increased (Table 1). The percentage of other internal body organs was much higher in the recently metamorphosed lamprey than in the other growth phases of the lamprey (Table 1).

The procedures used for compositional analysis accounted for 96.4–99.3% of the components in the body parts of 2-yr-starved and recently metamorphosed lampreys, respectively (Table 2). Lipid content was higher and soluble ash, sugars, and glycogen contents lower in the head than in the body sections of both the starved and metamorphosed lampreys. The

TABLE 1. Whole weight and percentage distribution of body parts of the 2-yr-starved, metamorphosed, and 6-mo-starved *L. tridentata*. N/S = not separated because gonad content minimal.

Lamprey (sex)	Wet weight (g)	Head (%)	Body (%)	Liver (%)[a]	Gonad (%)[b]	Remainder (%)
Starved 2 yr (m)	48.02	35.86	59.95	2.46	N/S	1.73
Metamorphosed (m)	1.92	35.14	47.52	1.20	N/S	16.14
Starved						
0 d (m)	43.48	33.39	62.26	1.43	2.18	0.74
0 d (f)	56.57	28.60	60.84	1.19	8.72	0.65
91 d (m)	20.87	32.44	61.52	2.35	2.25	1.44
91 d (f)	38.25	28.25	51.65	2.19	16.38	1.52
182 d (m)	19.28	37.81	57.00	2.91	1.35	0.93
182 d (m)	21.50	40.84	55.53	2.19	0.42	1.02

[a]Hepatosomatic ratio.
[b]Gonadosomatic ratio.

TABLE 2. Biochemical composition and energy content of body parts of a 2-yr-starved and recently metamorphosed *L. tridentata*. ND = not determined because of insufficient quantities.

	Parts of lamprey starved for 2 yr				Parts of metamorphosed lamprey[a]		
Parameter	Head	Body	Liver	Remainder	Head	Body	Remainder
Component (% wet weight)							
Moisture	86.1	84.8	72.3	80.9	81.7	82.6	86.8
Insoluble ash	0.06	0.04	ND	ND	0.01	0.06	ND
Soluble ash	0.50	0.68	ND	ND	0.70	0.73	ND
Lipids	1.37	1.21	16.52	11.37	4.95	4.68	1.67
Sugars	0.06	0.10	0.18	0.07	0.10	0.18	0.14
Glycogen	0.23	0.23	0.26	0.30	0.24	0.28	0.24
Protein	10.57	11.35	9.49	5.74	11.52	10.40	7.49
Caloric content of wet tissue ($kJ \cdot g^{-1}$)							
Lipid	0.48	0.43	5.82	4.01	1.74	1.65	0.59
Carbohydrate	0.05	0.06	0.08	0.07	0.06	0.08	0.07
Protein	1.90	2.04	1.71	1.03	2.07	1.87	1.35
Total	2.43	2.53	7.61	5.11	3.87	3.60	2.01
Energy in parts of a normalized 1-g lamprey (kJ)[b]	0.87	1.52	0.19	0.09	1.36	1.71	0.32

[a]Insufficient liver for complete analysis, although separated from other internal organs.
[b]Calculated from the caloric content of wet tissue and the corresponding percentage distribution of the body part in the lamprey from Table 1.

moisture, insoluble ash, and protein levels between the head and body parts were not consistently higher or lower in either the starved or metamorphosed lampreys. The 16 and 11% of lipid in the liver and remaining internal organs of the 2-yr-starved lamprey contrasted with the 2% in the internal organs of the metamorphosed lamprey. This resulted in a higher total caloric value for the internal organs of the starved lamprey.

Higher moisture levels and lower soluble ash, lipid, and carbohydrates in the head and body sections of the 2-yr-starved lamprey relative to the metamorphosed lamprey were consistent with the prolonged period of starvation (Table 2). The total caloric content of the head and body of the metamorphosed lamprey was at least 1.2 $kJ \cdot g^{-1}$ higher than that of the starved lamprey, principally from higher content of lipid. With the weight of the 2-yr-starved and metamorphosed lampreys being so dissimilar, a normalized 1-g lamprey was used for energy comparisons. Total energy per 1 g of the 2-yr-starved and metamorphosed lampreys was 2.67 and 3.39 kJ. The more energetically favoured metamorphosed lamprey reflected a high storage of lipid in the head tissue (Table 2).

Fatty acid profiles of lipids in the head and body of the 2-yr-starved and metamorphosed lampreys were qualitatively similar, differing mainly in the 4% of 15:0 acid in the latter (Table 3). The starved lamprey exhibited higher percentages of 18:0, 18:1*n*9, 20:4*n*6, 20:5*n*3, 22:5*n*3, and 22:6*n*3 acids and lower percentages of 14:0 and 16:1*n*7 acids than the metamorphosed lamprey. Higher levels of 20:5*n*3 in the liver than in the body walls of both starved and metamorphosed lampreys suggested either enzymatic capability to synthesize this acid from lower carbon fatty acids or that this acid was available in freshwater organisms. The latter is more probable, as the unexpected 21% 22:6*n*3 in the other internal organs of the metamorphosed lamprey suggested a source from dietary freshwater organisms. Lower contents of saturated and monoethylenic fatty acids in the starved relative to the metamorphosed lampreys conferred to the former a high proportion of polyethylenic acids which were principally polyunsaturated *n*3 acids (Table 3).

To determine that the biochemical differences between the 2-yr-starved and metamorphosed lampreys were more a function of starvation than influences of the preceding trophic envi-

TABLE 3. Fatty acid composition (relative %) in the lipids of body parts of a 2-yr-starved and recently metamorphosed *L. tridentata*.

	Body parts of lamprey starved for 2 yr				Body parts of metamorphosed lamprey			
Fatty acid	Head	Body	Liver	Remainder	Head	Body	Liver	Remainder
12:0	0.20	0.20	0.23	0.31	2.54	3.69	0.21	0.23
14:0	1.12	0.52	0.61	0.79	15.73	15.42	6.33	2.97
15:0	—	—	—	—	3.59	4.44	0.93	0.67
16:0	12.90	15.15	5.61	25.11	12.77	12.40	12.88	13.78
16:1*n*9	0.24	0.23	0.24	0.25	0.21	0.20	0.88	0.32
16:1*n*7	3.79	2.82	1.69	9.46	24.01	23.77	12.88	8.42
16:3*n*4	0.20	0.20	0.23	0.50	4.92	4.94	0.86	1.81
16:4*n*1	1.93	2.40	0.90	0.59	—	—	—	—
18:0	5.91	7.40	4.09	4.24	2.33	2.17	5.45	6.72
18:1*n*9	15.90	17.78	31.00	29.47	14.63	13.53	25.49	11.38
18:1*n*7	3.87	4.16	4.02	7.04	2.43	1.99	3.83	3.87
18:2*n*9	0.69	0.78	0.24	0.48	0.28	0.29	0.27	0.20
18:2*n*6	0.77	1.29	0.38	0.59	0.47	0.48	0.42	0.70
20:1*n*11	0.34	0.43	0.90	0.20	0.26	0.25	0.46	3.46
20:1*n*9	0.56	0.58	1.61	0.43	0.33	0.29	0.47	1.28
20:2h	0.64	0.73	0.56	0.36	0.34	0.47	0.93	0.50
20:4*n*6	6.35	6.57	3.90	2.52	0.75	0.98	1.85	2.18
20:4*n*3	0.36	0.31	1.58	0.28	0.21	0.22	0.21	0.30
20:5*n*3	12.36	12.33	20.30	1.96	1.36	1.43	5.31	7.02
22:4*n*6	0.46	0.44	1.20	0.28	0.20	0.25	0.23	0.23
22:5*n*3	3.09	1.69	4.49	0.82	0.66	0.73	1.67	2.92
22:6*n*3	19.71	15.03	9.47	6.27	2.85	2.71	9.69	21.06
Saturated	20.13	23.27	10.54	30.45	36.96	38.12	25.80	24.37
Monoethylenic	24.70	26.00	39.46	46.85	41.87	40.03	44.01	28.73
Polyethylenic	46.56	41.77	43.25	14.65	12.04	12.50	21.44	36.92
Σ4-6,*n*3/poly.	76.29	70.29	82.87	63.69	42.19	40.72	78.73	84.78
Σ4-6,*n*6/poly.	14.63	7.70	11.79	19.11	7.89	9.84	9.70	6.53

ronment, other specimens of upstream-migrating lampreys were analyzed over a 6-mo holding period (Table 4). The variation in composition of head and body sections of the male lampreys after 182 d was greater than those demonstrated between male and female lampreys taken earlier in the starvation period (Table 4). Other studies on lampreys have shown no significant differences between components in body tissue of males and females at the same stage of development (Bird and Potter 1983b). Linear regression was therefore fitted only to the data from head and body tissue irrespective of gender.

Starvation of lampreys in the laboratory for 182 d resulted in a linear increase in the moisture content in the head by $0.0354\%\cdot d^{-1}$ ($r^2 = 0.8711$) and in the body by $0.0387\%\cdot d^{-1}$ ($r^2 = 0.9368$). Insoluble ash as a minor component in head and body of lampreys averaged 0.05 and 0.07% wet weight, respectively, and showed no linear trend. Conversely, soluble ash decreased linearly in starved head and body tissue by $0.0028\%\cdot d^{-1}$ ($r^2 = 0.7235$) and $0.0033\%\cdot d^{-1}$ ($r^2 = 0.7177$), respectively. Lipid in the head of the lamprey was catabolized linearly at $0.0426\%\cdot d^{-1}$ ($r^2 = 0.9038$) and in the body at $0.0451\%\cdot d^{-1}$ ($r^2 = 0.8718$). No consistent loss in low molecular weight or polymeric carbohydrate was evident with starvation of the lamprey. Protein in the head and body was catabolized linearly during starvation at $0.0187\%\cdot d^{-1}$ ($r^2 = 0.8108$) and $0.0153\%\cdot d^{-1}$ ($r^2 = 0.8588$), respectively.

Substantial differences in the composition of internal organs of male and female lampreys resulted from starvation (Table 4). Lipid was stored to a greater extent in the liver of males than in females and the converse for gonadal tissue. Lipid content was higher in the tissue of liver than in all other body parts of the lamprey, but carbohydrate and protein levels were quite similar (Table 4). Lipid was also higher in ovaries than in the other parts of the lamprey.

The caloric content of lipid in the head and body tissue declined linearly with time of starvation, $0.0094\ kJ\cdot g^{-1}\cdot d^{-1}$ ($r^2 = 0.9022$) and $0.0098\ kJ\cdot g^{-1}\cdot d^{-1}$ ($r^2 = 0.8752$), respectively. A linear decline in the caloric content of protein in the head tissue, $0.0132\ kJ\cdot g^{-1}\cdot d^{-1}$ ($r^2 = 0.9270$), and body tissue, $0.0131\ kJ\cdot g^{-1}\cdot d^{-1}$ ($r^2 = 0.9181$), occurred with starvation. Caloric content of carbohydrate in the head and body tissue was reasonably constant at $0.11\ kJ\cdot g^{-1}$ (SD ± 0.04) and $0.11\ kJ\cdot g^{-1}$ (SD = ± 0.03), respectively. The caloric content of liver and gonad tissue fluctuated during starvation.

Energy derived from lipid and protein in a normalized 1-g lamprey declined with starvation at $0.0101\ kJ\cdot d^{-1}$ ($r^2 = 0.7878$) and $0.0040\ kJ\cdot d^{-1}$ ($r^2 = 0.8320$), respectively. Energy from carbohydrate was constant at 0.10 kJ (SD ± 0.03). Total energy required to maintain a 1-g lamprey during 6 mo of starvation was 2.56 kJ or 49% of the starting energy (whole energy $d_{(0-182)} = 5.2725 - 0.0141\cdot d$ ($r^2 = 0.7970$)). The total energy lost was derived 71% from lipid and 29% from protein catabolism.

Differences in relative proportions of fatty acids in tissue of male and female lampreys, other than gonads, were minor and mean values are presented in Table 5 for 0-, 91-, and 182-d specimens. Starvation caused a decrease in 14:0 and 16:1*n*7 acids in all tissues and a decline in 18:1*n*9 only in the head and body tissue, presumably as energy sources. The percentage of 18:0, 20:4*n*6, 20:5*n*3, 22:5*n*3, and 22:6*n*3 acids increased in the head and body, and 18:1*n*9 and 20:5*n*3 increased in the liver with prolonged starvation. The remaining internal organs, excluding gonads, showed a general increase in 16:0, 18:0,

TABLE 4. Biochemical composition, caloric content of body parts, and energy in *L. tridentata* held in fresh water under starvation conditions for 182 d.[a] ND = not determined because of insufficient quantities.

Parameter	Starved 0 d		Starved 91 d		Starved 182 d	
	Male	Female	Male	Female	Male	Male
Component (% wet weight)						
Moisture						
Head	77.1	76.8	83.8	80.5	84.2	87.0**
Body	75.2	74.8	81.2	79.1	83.4	86.1***
Liver	61.3	75.9	67.5	76.0	67.9	71.3
Gonad	84.2	53.6	ND	56.6	ND	ND
Insoluble ash[b]						
Head	0.05	0.03	0.07	0.06	0.03	0.03
Body	0.01	0.08	0.08	0.05	0.11	0.08
Soluble ash[b]						
Head	0.74	0.82	0.64	0.65	0.65	0.61*
Body	0.99	0.94	0.82	0.79	0.82	0.78*
Lipids						
Head	6.42	6.10	2.26	4.20	1.69	1.09***
Body	6.63	5.99	2.37	4.92	1.58	0.90**
Liver	26.24	8.76	19.00	6.91	19.61	15.74
Gonad	4.37	18.42	ND	15.22	ND	ND
Sugars						
Head	0.10	0.11	0.06	0.07	0.10	0.08
Body	0.27	0.27	0.15	0.22	0.11	0.12***
Liver	0.21	0.11	0.10	0.15	0.25	0.23
Gonad	0.10	0.10	ND	0.43	ND	ND
Glycogen						
Head	0.40	0.37	0.41	0.84	0.68	0.29
Body	0.37	0.27	0.48	0.55	0.60	0.19
Liver	0.51	0.65	0.37	1.00	0.41	0.37
Gonad	0.34	0.43	ND	0.50	ND	ND
Protein						
Head	13.11	14.55	12.38	11.60	10.97	9.02**
Body	15.27	14.86	13.18	12.32	12.37	11.15**
Liver	11.37	14.26	12.84	15.60	11.31	11.39
Gonad	10.00	24.62	ND	25.20	ND	ND
Caloric content of wet tissue ($kJ \cdot g^{-1}$) (energy in a normalized 1 g lamprey (kJ)[c])						
Lipid						
Head	2.26 (0.75)	2.15 (0.61)	0.80 (0.26)	1.48 (0.42)	0.60 (0.23)	0.38 (0.16)*** (***)
Body	2.34 (1.46)	2.11 (1.28)	0.84 (0.52)	1.73 (0.89)	0.56 (0.32)	0.32 (0.18)** (***)
Liver	9.25 (0.13)	3.09 (0.04)	6.70 (0.16)	2.44 (0.05)	6.91 (0.20)	5.55 (0.12)
Gonad	1.54 (0.03)	6.49 (0.57)	ND ND	5.36 (0.88)	ND ND	ND ND
Whole	— (2.37)	— (2.50)	— (0.94)	— (2.24)	— (0.75)	— (0.46)(***)
Carbohydrate						
Head	0.09 (0.03)	0.09 (0.03)	0.09 (0.03)	0.17 (0.05)	0.15 (0.06)	0.07 (0.03)
Body	0.12 (0.07)	0.10 (0.06)	0.12 (0.07)	0.14 (0.07)	0.13 (0.07)	0.06 (0.03)
Liver	0.13 (0.00)	0.14 (0.00)	0.09 (0.00)	0.22 (0.01)	0.12 (0.00)	0.11 (0.00)
Gonad	0.08 (0.00)	0.10 (0.01)	ND ND	0.17 (0.03)	ND ND	ND ND
Whole	— (0.10)	— (0.10)	— (0.10)	— (0.16)	— (0.13)	— (0.06)
Protein						
Head	2.36 (0.79)	2.62 (0.75)	2.23 (0.72)	2.09 (0.59)	1.97 (0.74)	1.62 (0.66)**
Body	2.75 (1.71)	2.67 (1.62)	2.37 (1.46)	2.22 (1.15)	2.23 (1.27)	2.01 (1.12)** (**)
Liver	2.05 (0.03)	2.57 (0.03)	2.31 (0.05)	2.81 (0.06)	2.04 (0.06)	2.05 (0.05)(**)
Gonad	1.80 (0.04)	4.43 (0.39)	ND ND	4.54 (0.74)	ND ND	ND ND
Whole	— (2.57)	— (2.79)	— (2.23)	— (2.54)	— (2.07)	— (1.83)(***)
Total						
Head	4.71 (1.57)	4.86 (1.39)	3.12 (1.01)	3.74 (1.06)	2.72 (1.03)	2.07 (0.85)*** (***)
Body	5.21 (3.24)	4.88 (2.97)	3.33 (2.05)	4.09 (2.11)	2.92 (1.66)	2.39 (1.33)*** (***)
Liver	11.43 (0.16)	5.80 (0.07)	9.10 (0.21)	5.47 (0.12)	9.07 (0.26)	7.71 (0.17)
Gonad	3.42 (0.07)	11.02 (0.96)	ND ND	10.07 (1.65)	ND ND	ND ND
Whole	— (5.04)	— (5.39)	— (3.27)	— (4.94)	— (2.95)	— (2.35)(***)

[a]Linear regression fitted to the data provided levels of significance: $*p < 0.05$; $**p < 0.02$; $***p < 0.005$ (levels in parentheses correspond to data in parentheses).

[b]Insufficient liver and gonad for determination of ash contents.

[c]Calculated from the caloric content of wet tissue and the corresponding percentage distribution of the body part in the lamprey from Table 1.

TABLE 5. Fatty acid composition (relative %) in the lipids of body parts of *L. tridentata* held in the laboratory under conditions of food deprivation for 182 d.

	Head, days starved			Body, days starved			Liver, days starved			Remainder, days starved[a]		
Fatty acid	0	91	182	0	91	182	0	91	182	0	91	182
12:0	1.61	1.54	1.21	1.54	1.81	0.65	0.38	0.21	0.20	0.65	0.34	0.20
14:0	11.79	11.66	8.27	9.89	11.42	1.55	5.54	5.23	2.19	6.27	3.33	1.63
16:0	13.65	10.74	10.53	14.23	10.76	13.38	12.59	8.38	2.97	16.54	22.35	21.15
16:1*n*9	0.33	0.20	0.75	0.33	0.21	0.31	0.31	0.28	0.32	0.53	0.21	0.74
16:1*n*7	16.02	17.40	12.82	16.08	17.13	3.93	9.48	9.39	4.55	11.90	8.57	6.07
16:3*n*4	0.22	0.24	0.20	0.22	0.20	0.20	0.23	0.21	0.20	0.20	0.30	0.21
18:0	2.13	3.50	5.16	2.68	3.22	7.82	2.95	1.87	3.48	3.52	5.30	7.33
18:1*n*9	24.90	22.91	19.86	23.41	23.99	17.59	29.49	33.34	35.58	21.96	23.83	21.64
18:1*n*7	3.16	3.03	3.09	3.07	2.91	4.24	3.95	3.59	3.52	3.57	4.98	4.82
18:2*n*9	0.30	0.71	0.33	0.32	0.61	0.64	0.24	1.30	0.29	0.45	0.63	0.21
18:2*n*6	0.55	0.34	0.30	0.63	0.42	0.75	0.43	0.25	0.25	0.91	0.27	0.35
20:1*n*11	0.64	0.24	0.20	0.84	0.90	1.12	0.34	0.68	1.67	0.67	0.32	0.35
20:1*n*9	0.83	0.48	0.25	0.91	0.49	1.04	0.64	0.91	1.68	0.66	0.43	0.57
20:2h	—	0.79	0.94	0.51	0.73	0.45	1.54	1.65	1.00	0.51	1.13	0.84
20:4*n*6	1.10	2.66	3.18	1.49	2.41	6.70	2.22	3.42	3.36	3.02	4.27	5.48
20:4*n*3	0.38	0.26	0.20	0.41	0.28	0.23	0.20	0.30	0.70	0.50	0.20	0.23
20:5*n*3	5.85	4.89	7.01	5.97	4.55	10.95	7.25	6.32	17.37	6.86	4.02	7.43
22:4*n*6	0.20	0.20	0.20	0.64	0.20	0.37	0.43	0.37	0.45	0.37	0.54	0.46
22:5*n*3	1.76	1.77	1.81	1.88	1.87	2.25	3.56	4.31	3.39	2.73	2.24	2.34
22:6*n*3	9.47	9.16	16.06	11.01	10.72	17.36	12.91	11.32	11.80	12.07	6.92	9.48
Saturated	29.18	27.44	25.17	28.34	27.21	23.40	21.46	15.69	8.84	26.98	31.32	30.31
Monoethylenic	45.88	44.26	36.97	44.64	45.63	28.23	44.21	48.19	47.32	39.29	38.34	34.19
Polyethylenic	19.83	21.02	30.23	23.08	21.99	39.90	29.01	29.45	38.81	27.62	20.52	27.03
Σ4-6,*n*3/poly.	88.05	76.50	82.96	83.49	79.22	77.17	82.45	75.55	85.70	80.23	65.21	72.09
Σ4-6,*n*6/poly.	6.56	13.61	11.18	9.22	11.87	17.72	9.13	12.87	9.82	12.27	23.44	21.98

[a]Excluding gonads, values of which are presented in Table 6.

20:4*n*6, and 20:5*n*3 acids and a decline in the 22:6*n*3 acid. Saturated fatty acids decreased in head, body, and liver tissue but increased in the other internal organs with progressive starvation. The monoethylenic acids declined in the head and body but increased in the liver. The polyethylenic acids increased in head, body, and liver during starvation but mainly from an increase in polyunsaturated *n*6 acids (Table 5).

It would seem prudent for prespawning lampreys not to rob their gonads of energy, thereby ensuring the quality and viability of sperm and eggs. Changes in relative proportions of fatty acids with starvation were more pronounced in testes than in ovaries (Table 6). In the testes the decline in content of 14:0 and 16:0 acids may have resulted from their conversion to, and increase in, the 16:1*n*7 acid. The use of this acid as a source of maintenance energy in the lamprey was alluded to previously. The eicosanoid hormone precursor 20:4*n*6 increased substantially over the 6-mo period in the testes and the content of 18:2*n*6, one of its precursors, declined. It is feasible that this acid could be an indicator of testes maturation in lampreys. The decline in saturated acids in the testes was compensated for by an increase in polyethylenic acids, principally the 20:4*n*6 acid (Table 6). In comparison with the testes, the ovaries showed remarkable stability to starvation, with little change in combined classes of acids (Table 6). The minor decline in 14:0 and 16:0 acids was compensated for by a corresponding increase in the 18:1*n*9 acid, presumably from the elongation and desaturation of the saturated acids. A conservative anabolic rearrangement of relative proportions of fatty acids in the gonads, rather than any catabolic loss, therefore appeared to have occurred during the starvation of the Pacific lamprey.

Discussion

Adult *L. tridentata* migrate into the Stamp River from May to September, with the largest number migrating in July (Beamish 1980). It is probable that the lampreys caught in October and subjected to 6 mo of starvation in the laboratory were caught about 3 mo after they had entered the Somass River. This additional starvation period, when applied to the linear regression equation derived for weight loss on starvation, suggested that the lampreys entered the river at about 63 g. The increase from a 2-g metamorphosed lamprey to an approximately 63-g adult reentering the river is similar to the growth of *L. fluviatilis* which increases from 1–2 g as a downstream migrant to 53 g as an adult after a marine trophic stage of 18 mo (Hardisty and Potter 1971; Abou-Seedo and Potter 1979; Bird and Potter 1979). Calculations for the 2-yr-starved lamprey, which had been caught almost immediately on reentry into the river in June, suggested a reentry weight of about 167 g and a considerably longer trophic phase of development in salt water, as has been suggested previously by Beamish (1980). The higher weight gain for this lamprey is similar to that of anadromous *P. marinus* and *G. australis*, which increase from their downstream migrant weights by about 290 times during the parasitic phases of 23–28 mo (Potter and Beamish 1977; Beamish et al. 1979). The gonadosomatic ratios for *L. tridentata* were similar to the 10.02 and 4.57% recorded for female and male *L. fluviatilis*, and although the hepatosomatic ratio of the Pacific lamprey increased slightly during starvation, values were similar to the 2.02 and 1.29% recorded for female and male *L. fluviatilis* (Heikkala et al. 1984).

TABLE 6. Fatty acid composition (relative %) in the lipids of gonads of *L. tridentata* held in the laboratory under conditions of food deprivation.

	Testes			Ovaries	
Fatty acid	Before starvation	Starved 91 d	Starved 182 d	Before starvation	Starved 91 d
12:0	0.40	0.20	0.20	0.20	0.20
14:0	5.46	2.91	1.49	6.05	5.66
16:0	14.77	9.07	8.21	15.08	13.15
16:1*n*9	0.31	0.24	0.31	0.20	0.20
16:1*n*7	10.66	12.74	15.94	11.78	10.43
16:3*n*4	0.20	0.21	0.20	0.31	0.24
18:0	3.33	7.36	4.72	3.04	3.00
18:1*n*9	24.77	17.63	19.74	17.12	20.07
18:1*n*7	4.83	4.99	4.57	2.63	3.72
18:2*n*9	0.25	0.44	0.21	0.56	0.62
18:2*n*6	0.86	0.21	0.30	0.47	0.40
20:1*n*11	0.46	0.47	0.40	0.39	0.20
20:1*n*9	0.63	0.58	0.50	0.41	0.34
20:2h	0.23	1.79	1.28	0.35	0.43
20:4*n*6	0 20	4.95	5.32	1.92	1.79
20:4*n*3	0.46	0.20	0.20	0.52	0.20
20:5*n*3	7.28	3.52	6.04	10.47	11.05
22:4*n*6	0.23	0.32	0.35	0.20	0.22
22:5*n*3	2.31	2.30	2.82	3.61	2.76
22:6*n*3	13.79	20.10	19.82	19.44	19.11
Saturated	23.96	19.54	14.62	24.37	22.01
Monoethylenic	41.66	36.65	41.46	32.53	34.96
Polyethylenic	25.81	34.04	36.54	37.85	36.82
Σ4-6,*n*3/poly.	92.37	76.76	79.04	89.93	89.95
Σ4-6,*n*6/poly.	1.67	15.48	15.51	5.60	5.45

Biochemical differences between the 2-yr-starved and metamorphosed lampreys reflected the extent of food deprivation. Increased hydration of tissue in the 2-yr-starved lamprey, relative to the metamorphosed lamprey, is a common function of starvation in many species (Mayzaud 1976; Taylor and Venn 1979; Cuzon et al. 1980; Beninger and Lucas 1984; Whyte et al. 1986; Whyte et al. 1990). The soluble ash content declined with starvation. The generally low ash content in *L. tridentata* was similar to the 0.8% reported in *P. marinus* and *G. australis* but about four times that found for *I. gagei* (Beamish et al. 1979; Bird and Potter 1981; Beamish and Legrow 1983). The glycogen content was constant in all tissue from the starved and metamorphosed lamprey, but the minor yet variable content of sugars suggested a constant metabolic use of the low molecular weight carbohydrate pool throughout starvation. In the citric acid cycle, carbohydrate is the main source of oxaloacetate, an intermediate in the cycle essential for complete oxidation of lipid. Although comprising 0.5% or less of the total tissue, carbohydrate was maintained constant for metabolic processes in starved *L. tridentata*, as in other lampreys during nontrophic phases (Beamish et al. 1979).

The protein level in recently metamorphosed Pacific lamprey, about 11%, was similar to the 12–13% level found in *P. marinus* during metamorphosis (Lowe et al. 1973). During metamorphosis, anabolic processes for protein formation must provide for enlargement of fins, eyes, suctorial disc, teeth, and tonguelike piston (Hardisty and Potter 1971). Any catabolism of protein as an energy source during this anatomical rearrangement would not be metabolically expedient.

The fatty acid profiles of the 2-yr starved and metamorphosed lampreys differed qualitatively in the presence of 16:1*n*4 in the former and 15:0 in the latter. The odd-carbon saturated acid was also exclusive to the larval stages of *G. australis* and is apparently derived from a freshwater organism rich in this acid (Bird and Potter 1983a). The decrease in 14:0 and 16:1*n*7 acids, particularly in the body, and the lack of change in appropriate higher isomers suggest their oxidation as major energy sources during starvation.

Hydration occurred in the lamprey tissue during 6 mo of starvation, similar to the increased hydration noted between the 2-yr-starved and metamorphosed lampreys. Increase of moisture with starvation is normally accompanied in marine species by the uptake of chloride salts to compensate for ionic imbalance (Whyte et al. 1990). In fresh water with low elemental concentration, ion uptake is less probable. A decline in plasma sodium was reported previously in starved *L. tridentata* by Clarke and Beamish (1987). The 2-yr-starved lamprey had 0.5–0.7% soluble ash as opposed to 0.7–1.0% in the 6-mo-starved lampreys and suggested that cellular ionic imbalance may be the ultimate cause of death.

In the head and body of the lamprey starved for 6 mo, a significant linear decline in caloric content derived from lipid and protein occurred, but the carbohydrate content remained constant. These changes were reflected in the differences between the 2-yr-starved and metamorphosed lampreys. Values reported by Beamish and Legrow (1983) for the caloric content of the carcass of male and female adult *I. gagei* when recalculated for wet tissue gave 4.30 and 6.01 $kJ \cdot g^{-1}$, respectively, in close agreement with the 4.71–5.21 $kJ \cdot g^{-1}$ for the head and body tissue of male and female *L. tridentata*. As Beamish and Legrow (1983) in their study on *I. gagei* did not provide the moisture content of the liver and gonads, the caloric contents of these organs in the Pacific lamprey were recalculated (as kilocalories) on a dry weight basis. Liver and gonads of male

and female Pacific lampreys contained 7.06 and 5.75 and 5.17 and 5.68 kcal·g dry tissue^{-1}, respectively. In reasonable agreement were the 6.13 and 6.05 and 5.37 and 6.62 kcal·g dry weight^{-1} of the liver and gonads in the male and female *I. gagei* (Beamish and Legrow 1983). The energy loss from linear regression analysis of starved lamprey on a wet tissue basis was 0.0141 kJ·g^{-1}·d^{-1}, derived 71% from lipid and 29% from protein catabolism. The average 2.66 kJ·g wet tissue^{-1} of the 6-mo-starved lamprey, in reality starved for about 9 mo from entering the river, and 2.67 kJ·g^{-1} in the 2-yr-starved lamprey was slightly higher than the average 2.35 kJ·g wet weight^{-1} reported by Beamish and Legrow (1983) for spent adult *I. gagei*. The recently metamorphosed male Pacific lamprey had an energy level of 3.39 kJ·g wet weight^{-1}, in contrast with the 5.50 kJ·g^{-1} reported for metamorphosed male *I. gagei* (Beamish and Legrow 1983).

The decrease or increase in the relative proportions of the fatty acids between the 2-yr-starved and metamorphosed lampreys was reflected in changes of corresponding fatty acids in the 6-mo-starved lampreys. Both 14:0 and 16:1*n*7 were used as energy sources in the lamprey; the latter acid is a major constituent of marine and freshwater fish (Ackman 1967; Whyte 1988). The decline with starvation, in an otherwise high content of 18:1*n*9, particularly in the enzymatically active liver, indicates the inability of the cartilagenous lamprey to convert this acid readily to 18:2*n*6, as is the case with most vertebrates. The decline in content of C-14 and C-16 fatty acids on starvation resulted in an increase in the C-20 and C-22 polyunsaturated isomers, which originate largely from marine particulate matter (Mayzaud et al. 1989). The presence of arachidonic acid, 20:4*n*6, in substantial amounts in all body parts of the lamprey suggests its requirement in the formulation of pharmacologically active eicosanoids, such as prostaglandins and thromboxanes, which regulate many different cell functions (Ruggeri and Thoroughgood 1985). Bird and Potter (1983a) reported 6–8% 20:4*n*6 in the phospholipid fraction of *G. australis*. Both eicosapentaenoic, 20:5*n*3, and docosahexaenoic, 22:6*n*3, acids, which are common to most marine species, have been regarded as nutritionally essential for growth and to maintain membrane fluidity and permeability characteristics in cold-water environments (Castell 1970; Watanabe et al. 1983). The buildup of these acids in the body wall of the starved Pacific lamprey and their high content in phospholipids of *G. australis* (Bird and Potter 1983a) tend to support a structural role in the cartilaginous lamprey, which, unlike skeletal fish, can shrink in size and slowly catabolize tissue for maintenance energy.

The longest recorded period of survival of a fish deprived of food is 3.5 to just over 4 yr for the African lungfish (*Protopterus annectens*) (Coates 1937; Smith 1939; El Hakeem 1979). African lungfish aestivate generally for 9 mo in underground cocoons in dry river beds in Central Africa during the dry season. The ability to slowly catabolize its tissue for maintenance energy is an adaptive physiology to ensure its survival should the dry season be prolonged (El Hakeem 1979). The ability of lampreys in general, and the Pacific lamprey in particular, to survive prolonged periods without feeding must also be a natural adaptation for survival. In northern British Columbia, the Pacific lamprey migrates 402 km up the Skeena River into Babine Lake where spawning lampreys have been deprived of food for about 14 mo (Farlinger and Beamish 1984). Spawning in the headwaters of large rivers allows for distribution of the larval lamprey throughout the watershed and access to vast areas of rearing habitat.

Present-day lampreys are presumably descendants of primitive lampreys that existed in the Carboniferous period at least 300 million yr ago (Janvier and Lund 1983). During the later Permian period, about 230 million yr ago, the union of the continents to form Pangaea drained shallow seas, exposed continental shelves, and reduced favoured marine environments, resulting in the extinction of many marine species. The ability of lamprey to withstand prolonged periods of starvation by using endogenous reserves may have been an important adaptation to survival during this later Palaeozoic period, which now allows it to survive extended periods of nontrophic development.

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Smolt-to-Adult Survival Patterns of Sockeye Salmon (*Oncorhynchus nerka*): Effects of Smolt Length and Geographic Latitude when Entering the Sea

Jeffery P. Koenings and Harold J. Geiger

Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, P.O. Box 25526, Juneau, AK 99802, USA

and James J. Hasbrouck

Alaska Department of Fish and Game, Sport Fish Division, 333 Raspberry Road, Anchorage, AK 99518, USA

Koenings, J. P., H. J. Geiger, and J. J. Hasbrouck. 1993. Smolt-to-adult survival patterns of sockeye salmon (*Oncorhynchus nerka*): effects of smolt length and geographic latitude when entering the sea. Can. J. Fish. Aquat. Sci. 50: 600–611.

Variations in smolt-to-adult survival (SAS) of sockeye salmon (*Oncorhynchus nerka*) relative to smolt length and age and latitude of the nursery lake outlet were explored for six stocks in Canada, Russia, and Alaska (W. E. Ricker. 1962. J. Fish. Res. Board Can. 19: 531–560) and 12 Alaskan populations. SAS values ranged from <1 to 68% for age 1., 2., and 3. smolt populations of 54–200 mm in mean length. The common pattern underlying the SAS to smolt size relationship, determined by nonparametric regression (loess), was nonlinear with functionally distinct zones. SAS of smaller smolts increased (0.3–0.5 (%) SAS·mm^{-1}) with size, but SAS of smolts >90–100 mm (6–8 g) did not. Variation in SAS was explained by smolt size (30%) and a south to north cline of nursery lakes (21%) indexed by latitude. SAS of about 3 and 13% for threshold-sized age 1. smolts (60–65 mm) from southern and northern nursery lakes were consistent with sockeye biostandards for Canada (4.7%) and Alaska (12%), respectively. The combined effects of latitude and smolt length on SAS resulted in larger smolts at higher (>60°N) latitudes having seven times the SAS of smaller smolts at lower (<55°N) latitudes.

Chez six stocks trouvés au Canada, en Russie et en Alaska et qui ont déjà été analysés par W. E. Ricker (1962. J. can. sci. halieut. aquat. 19 : 531–560), ainsi que chez 12 populations d'Alaska, on a examiné les variations de la survie du saumoneau à l'adulte (SSA) chez le saumon rouge (*Oncorhynchus nerka*) en fonction de la longueur et de l'âge des saumoneaux, ainsi que de l'emplacement de l'exutoire du lac d'alevinage. La SSA variait entre moins de 1 et 68 % dans les populations de saumoneaux mesurant 54–200 mm de longueur moyenne et âgés de 1., 2. et 3. ans. Telle que déterminée par une régression non paramétrique (loess), l'allure commune de la relation entre la SSA et la taille des saumoneaux était non linéaire et comportait des zones fonctionnellement distinctes. La SSA des plus petits saumoneaux s'accroissait proportionnellement à la taille (0,3–0,5 (%) SSA·mm^{-1}), mais ce n'était pas le cas de la SSA des saumoneaux de plus de 90–100 mm (6–8 g). Les variations de la SSA s'expliquaient par la taille des saumoneaux (30 %) et l'existence d'un clivage, orienté du sud au nord, des lacs d'alevinage (21 %) exprimé en fonction de la latitude. La SSA d'environ 3 et 13 % chez les saumoneaux de taille minimale et âgés de 1. an (60–65 mm) qui provenaient des lacs d'alevinage du sud et du nord était conforme aux standards biologiques du saumon rouge au Canada (4,7 %) et en Alaska (12 %), respectivement. L'effet combiné de la latitude et de la longueur des saumoneaux sur la SSA explique que les plus gros saumoneaux des hautes latitudes (>60 °N) ont une SSA sept fois supérieure à celle des petits saumoneaux à basses latitudes (<55 °N).

Received September 9, 1991
Accepted September 3, 1992
(JB211)

Reçu le 9 septembre 1991
Accepté le 3 septembre 1992

Although Foerster (1954) developed size-dependent smolt-to-adult survival (SAS) for Cultus Lake sockeye salmon (*Oncorhynchus nerka*), Ricker (1962) generalized the concept by integrating a wide range of smolt sizes from stocks in Canada, Alaska, and Russia. Thirty years later, Ricker's (1962) scatter plot is still the cynosure of sockeye SAS appearing intact in reviews of sockeye biology by Foerster (1968) and Burgner (1991).

Within Ricker's (1962) analysis, however, smaller and younger smolts from southern Canadian stocks dominated the lower range in SAS. Larger and older smolts and greater SAS occurred for more northern Alaskan and Russian stocks. Later, Koenings and Burkett (1987) postulated higher SAS, at the smaller smolt sizes, for Alaskan sockeye compared with more southern stocks in Ricker (1962). Thus, changes in SAS were consistent with broad shifts in both latitude and smolt size and age.

While most biologists argue that SAS of sockeye depend to some degree on smolt size, the overall pattern and magnitude of survival per length increment remains questionable, e.g. the SAS biostandards of Alaska (12%) versus Canada (4.7%). The broad effects of geographic range (Alaska to California) on SAS of coho salmon (*Oncorhynchus kisutch*) has been suggested (Bilton et al. 1982), with a south to north cline (Drucker 1972)

of increasing SAS per unit size explored by Hopley (1991). Changes in photoperiod and temperature scaled by latitude (Bilton et al. 1982) may augment or reduce SAS as well as seasonally synchronizing the outmigration timing of both sockeye (Harman et al. 1967) and coho (Drucker 1972) smolts to a seasonally pulsed marine food supply.

In addition, while sockeye smolt mean population lengths range between 54 and 200 mm, at some intermediate point in length, SAS probably begin to either plateau or decline, perhaps after a SAS size-advantage threshold is reached (Koenings and Burkett 1987). Similar or equal SAS for large and small smolts would further confound a simple size-dependent SAS relationship.

Circumstantial evidence of a limit to size-dependent SAS and a biological rationale for it does exist. First, Ricker (1962) limited size-related SAS at <107 mm, and Peterman (1981) found that Bristol Bay sockeye smolts of 110 mm had the least variable and highest SAS. Second, an intensive offshore seining study in the North Pacific only caught sockeye >100 mm (Hartt 1980) whereas 2 yr of nearshore seining in an Alaskan estuary only caught sockeye <100 mm (J. Orsi, NMFS, Auke Bay, AK, pers. comm.). SAS of nearshore rearing smolt populations <100 mm in size may differ in degree of size dependence from those of larger smolts that may go directly to sea.

We have assembled and explored all data available to us on the SAS of sockeye for underlying trends and to formulate new hypotheses about factors effecting SAS in salmon. We relied heavily on nonparametric exploratory data analysis tools (Efron and Tibshirani 1991) not available to Ricker (1962). First, we present recent SAS estimated for 12 Alaskan sockeye stocks and compare these with the data Ricker (1962) originally presented. Second, the effects of smolt size, age, and latitude and thresholds underlying SAS of sockeye are examined. Finally, we explore changes in SAS for discrete populations of sockeye having mean smolt sizes above and below 110 mm.

Methods and Materials

Historical sockeye SAS were taken from Ricker (1962) for Karluk Lake (smolt years 1926 and 1929–33), Dalneye Lake (1937–40 and 1945–49), and Babine Lake (1955–58). Also included were the SAS for Cultus Lake (1927–44) from Foerster (1954) after calculating total adult returns from escapement levels assuming a harvest rate of 66% (Ricker 1962). Bare Lake SAS for 1950–53 were taken from Nelson (1959), while Chilko Lake SAS for 1952–55 were derived from Parker (1962) and Foerster (1968). All nursery lakes were located in Canada except for Karluk and Bare lakes (Alaska) and Lake Dalneye (Russia). Age 1., 2., and 3. smolts are represented, but not all ages occurred in all stocks. The overall smolt sizes range from 63 to 169 mm.

Alaskan sockeye SAS are from nursery lakes located south of Ketchikan in Southeast Alaska to Prince William Sound, Cook Inlet, and Kodiak Island in Southcentral Alaska. Because of the diversity in data collection, details of the smolt and adult enumeration programs are briefly described for each system in Table 1. An unknown portion of the variation in SAS is caused by differences in the accuracy of the smolt and adult enumeration programs. The SAS generally cover the 1976–88 smolt years, but the SAS for Karluk Lake are for the 1962–66 smolt years. Age 1., 2., and 3. smolts are represented, but again not all ages occurred in all stocks. Although the overall range in smolt size was from 54 to 200 mm, only age 2. Hidden Lake smolts exceeded 170 mm. Exclusive of these fish the smolt sizes ranged up to 170 mm.

Overall, the estimates of smolt lengths represent population means derived from a number of weighted weekly periods covering the entire outmigration period. In general, smolts were anesthesized with MS-222, weighed to the nearest 0.1 g using a digital or analog balance, sized to the nearest 1 mm (fork length), and four scale smears per fish placed on glass slides. Ages were determined using magnified images from either a microfiche projector or microscope/video monitor after reading by two individuals. Adult enumerations covered the entire return period and were coupled, with varying degrees of accuracy involving stock separation (e.g. Marshall et al. 1987), to estimates of commercial, sport, and personal-use harvest rates. SAS were calculated by dividing the estimated number of age 1., 2., and 3. smolts into the total estimated numbers of the same freshwater (brood year) adults having varying ocean ages and converting to a percentage. Adult ages were determined from scales taken from adults passing through weirs, from fish wheel catches, and from commercial catches as part of stock separation programs.

Karluk Lake SAS (n = 12) for age 2. and 3. smolts from 1926 and 1929–33, also included in Ricker's (1962) data, were already corrected (1.22) for marking mortality based on results from similar marking studies on Babine and Cultus Lake sockeye. A second study of Karluk Lake sockeye included the 1962–66 period (Drucker 1970) during which estimates of total numbers of smolts were used to calculate SAS. Adult return data (n = 10 yr) were corrected for the location of the adult weir, although no significant difference existed between the adjusted adult returns and those in Drucker (1970).

Smolt sizes for Lake Washington sockeye were calculated using data from Eggers (1978) and J. Ames (Washington Department of Fisheries, Olympia, WA 98504, pers. comm.), while SAS were from Thorne and Ames (1987) and Hyatt et al. (1989). SAS for Lake Washington (Seattle) sockeye over the 1967–82 brood years (n = 14, as 1973–74 were not sampled) were recalculated using spring hydroacoustic estimates of presmolt densities (Thorne and Ames 1987; Hyatt et al. 1989). Burgner (1987) cited a mean size of 125 mm for Lake Washington age 1. smolts, Eggers (1978) cited a mean smolt size of 126.5 mm for the 1965–69 brood years, and Thorne and Ames (1987) cited a more recent smolt size of 120 mm. Presmolt lengths were available (J. Ames, pers. comm.) from fish captured using a midwater trawl for the 1967–68 (108.3–111.3 mm) and 1980–87 (97.2–108.6 mm) brood years. As presmolt lengths have slightly decreased over the 10 brood years, we used a comparison between the 1965–69 smolt sizes and the 1967–68 presmolt sizes (126.5 mm/110 mm or 1.15) to estimate a minimum size for age 1. smolts of ~117 mm from recent presmolt sizes. Thus, the actual smolt sizes cited by Burgner (1987) and Eggers (1978) of >125 mm during the 1960's may be closer to the 120 mm cited by Thorne and Ames (1987).

The Kvichak River sockeye smolt data (Woolington et al. 1991) were not included in the overall Alaskan data base, as smolts were indexed using a sonar counter. However, smolt counts along with weight and length data are consistently taken allowing comparison of interannual and intrasystem trends.

To define the underlying shape of the relationship between two variables, we used the nonparametric loess (robust locally weighted regression) technique (Cleveland 1979; Efron and Tibshirani 1991). The loess procedure employs locally weighted

TABLE 1. Brief descriptions of the sockeye smolt and adult enumeration programs at 12 nursery lakes located throughout Alaska. Adult stock separation (SS) techniques based on scale patterns were used to aportion harvested fish to system of origin.

Nursery system (smolt years)	Sockeye enumeration programs	
	Smolts	Adults
Hugh Smith (1981–82 and 1984–85)	Smolt fence and live box used for a total count over entire outmigration	Escapement by weir; commercial harvest using SS; personal use by permits; no sport harvest
McDonald (1983–86)	Spring hydroacoustic and tow-net survey corrected for holdovers using fall hydroacoustic surveys	Escapement by weir; commercial harvest using SS; personal use by permit; no sport harvest
Eshamy (1982–83)	Smolt fence and live box used for daily direct counts with biomass estimates or timed subsamples over entire outmigration	Escapement by weir; commercial harvest by SS and fish tickets; no personal-use or sport harvest
Tustumena (1981–85)	Six fan traps and holding boxes with mark–recapture (dye) to calibrate net efficiency weekly over entire outmigration	Escapement by side-scanning sonar; fish wheel for adult data; commercial harvest by SS; personal use and sport harvest by creel census
Crescent (1981)	Floating fan traps and holding boxes with mark–recapture (dye) to calibrate net efficiency for entire outmigration	Escapement by side-scanning sonar; fish wheel for adult data; commercial harvest by SS; no personal-use or sport harvest
Hidden (1976–84)	Fyke net and holding box that fished the entire stream; total count or timed subsample over entire outmigration	Escapement by weir; commercial harvest from mainstem Kenai River harvest rates; no personal-use or sport harvest
Russian (1978–79)	Smolt fence and live box using stratified random sampling for entire outmigration period; sonar aperature counter used with fence	Escapement by weir for early and late runs; commercial harvest for late run by mainstem Kenai River rates; sport harvest by creel census
Larson (1982)	Modified fyke net (with wings) that fished the entire stream; direct counts and timed samples from a live box over entireoutmigration	Escapement by weir; commercial harvest by SS using mainstem Susitna River rates; minor personal-use harvest and no sport harvest
Big (1985–88)	Fyke net and live box that fished the entire stream; direct counts or time subsampling over entire outmigration	Escapement by weir; commercial harvest by SS and modified Susitna River harvest rates; personal-use harvest by permit
Leisure (1979 and 1981–86)	Fyke net and live box that fished the entire stream direct counts or biomass estimates every hour over entire outmigration	No escapement; adults counted at Barrier near saltwater; commercial harvest by fish ticket; personal-use and sport harvest by biweekly survey
Packers (1981–84)	Modified fyke net (withwings) and live box that fished the entire stream; direct counts or biomass estimates every 6 h over entire outmigtation	Escapement by weir; commercial harvest by SS using harvest rates for nontargeted stocks in Cook Inlet; no personal-use or sport harvest
Karluk (1962–66)	Latin-square sampling design over entire outmigration (Drucker 1970)	Escapement by weir and adjusted for weir location; commercial harvest from catches in the Karluk District; river spawners below the weir; minor sport harvest

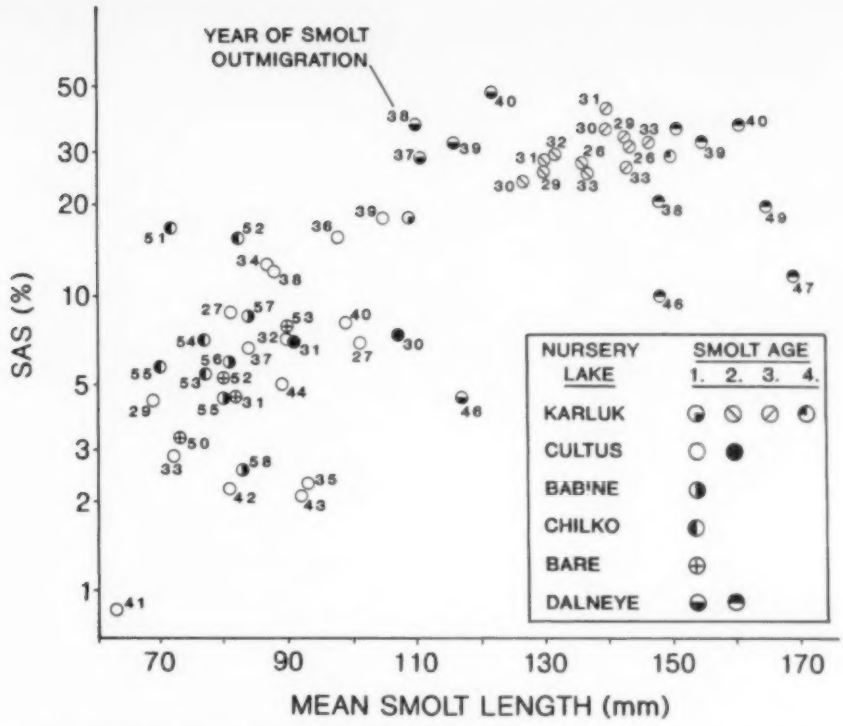


FIG. 1. Scatterplot relating SAS of sockeye to the mean length of age 1., 2., 3. and 4. smolts from three nursery systems located in Canada (Cultus, Chilko, Babine), two in Alaska (Karluk, Bare), and one in Russia (Dalneye) after Ricker (1962).

least squares to fit a line to a scatter plot without some of the constraints or biases of traditional linear regression. The weight ($F = 0.4$) of data points is estimated iteratively using robust estimation techniques. We also used Spearman's nonparametric correlation (rho value) to assess the relationship between two variables. Next, classical linear regression techniques (Draper and Smith 1981) were used to relate SAS to smolt size. One-way analysis of covariance (ANCOVA) was used to determine effects of either age or latitude on regression parameter estimates. In addition, two-way analysis of variance (ANOVA) was used to examine differences in SAS of recent Alaskan systems due to smolt age and length class and differences in SAS among all systems due to latitude and smolt length class. Tukey's multiple range test was used to determine differences in SAS associated with the incremental (10 mm) increases in smolt size class. Finally, differences between median values of SAS and smolt sizes were determined using the Mann–Whitney U-test.

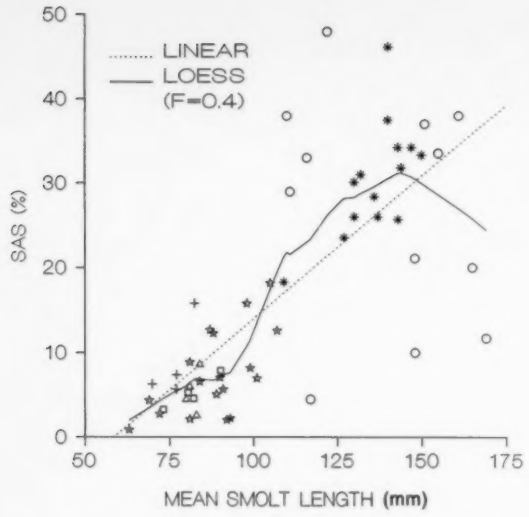
Results

Ricker's Sockeye SAS Patterns: Comparisons among Six Stocks

Sockeye SAS for six northern and southern stocks were analyzed for a relationship to smolt size (length) by Ricker (1962) and presented as a scatterplot graph without a numerical model (Fig. 1). There appeared to be a nonlinear relationship between the log of the percent SAS and smolt length. Given the statistical models available at the time, Ricker (1962) could only suggest the appearance of a break in the overall relationship beyond a smolt length of 107 mm (100–110 mm) where more northern stocks with age 2. and 3. smolts were clustered. Over the smolt length range of 63–169 mm, SAS ranged from a low of 0.85% for Cultus Lake to a high of 48% for Lake Dalneye stocks and covered smolt years from 1926 (Karluk) to 1958 (Babine).

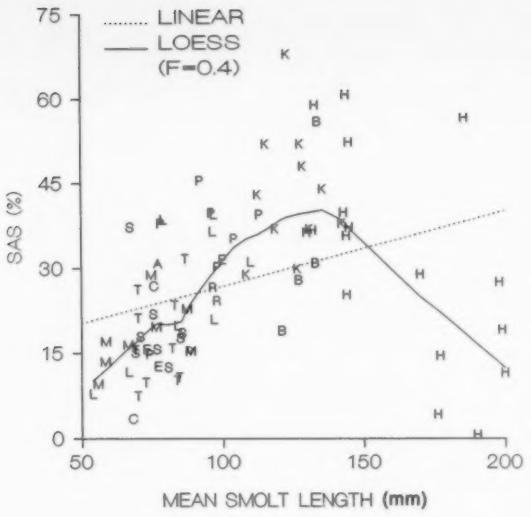
A further analysis of Ricker's (1962) data set occurred nearly 20 yr later when Hyatt and Stockner (1985) fit a curvilinear model to four ($n = 46$) of the six stocks plus two data points from Peterman (1981). We found no difference (ANCOVA; $P = 0.98$ and 0.99) in either the slope or mean response, respectively, between the four systems of Hyatt and Stockner (1985) and the six systems of Ricker (1962). Using an arcsine transformation, Hyatt and Stockner (1985) found that smolt length explained 58% of the variation in SAS, but more importantly applied a statistical model across the entire smolt size range.

Applying recently developed nonparametric (loess) regression techniques to Ricker's (1962) data ($n = 56$) revealed the basic curvilinear relationship (Fig. 2) between SAS and smolt length even though a linear model was significant. The loess pattern is also very similar to the "broken stick" pattern proposed for these data by Koenings and Burkett (1987). The arcing pattern suggests that increases in smolt length beyond a specified size result in smaller increases or even decreases in SAS.



□ BARE	☆ CULTUS	✱ KARLUK
△ BABINE	+ CHILKO	○ DALNEYE

FIG. 2. Linear and loess ($F = 0.4$) models relating SAS of sockeye to mean smolt lengths from six nursery systems in Canada, Alaska, and Russia from data originally presented by Ricker (1962) (see Fig. 1).



B BIG	H HIDDEN	M McDONALD
C CRESCENT	SH. SMITH	P PACKERS
E ESHAMY	A LARSON	T TUSTUMENA
K KARLUK	L LEISURE	R UP. RUSSIAN

FIG. 3. Linear and loess ($F = 0.4$) models relating SAS of sockeye to mean smolt lengths from 12 nursery systems located in Alaska.

Alaskan Sockeye SAS Patterns: Comparisons among 12 Stocks

SAS values ($n = 82$) taken from 12 northern sockeye stocks for the 1979–88 smolt years included estimates for age 1. and 2. smolts (Table 1). Also included are the previously unreported SAS values for Karluk Lake age 2. and 3. smolts from 1962 to 1966. Mean smolt lengths and SAS ranged from 54 to 200 mm and from 0.7 to 68%, respectively (Fig. 3). A linear model was significant, but had little explanatory power. In contrast, loess regression of SAS against smolt length results in a curvilinear relationship. As found for the Ricker (1962) data set, size-dependent SAS of Alaskan sockeye appear to exhibit an arc-shaped pattern.

SAS of age 1. smolts over the range of 54–145 mm generally increased with larger smolt lengths (rho = 0.57; $P < 0.01$). However, the loess regression for age 1. smolts formed a flattened, curvilinear pattern (Fig. 4). SAS increased from ~10 to 35% at smolt lengths between 55 and 90 mm, i.e. a 60% increase in smolt length caused a nearly fourfold increase in SAS (about 0.5 (%) SAS·mm^{-1}). SAS estimates for a threshold smolt size of 60 mm were about 13%, and little further survival advantage accrued to age 1. smolts larger than 90 mm. SAS of age 2. smolts also closely followed an arc-shaped pattern as defined by loess regression (Fig. 4). Comparing the loess regressions for the two age groups suggests that below 110 mm, age 2. smolts need be 10 or 15 mm larger to acquire the same SAS as age 1. smolts.

Smolts >170 mm were all age 2. fish from Hidden Lake ($n = 7$) and are the largest smolts reported in the sockeye literature (e.g. Burgner 1987). Because the influence of such large smolts on the SAS pattern (Fig. 3) may surpass their general appearance within smolt populations, we removed them from the analysis. This also produced a smolt size range more consistent with Ricker's (1962) data set. Even though a linear model is significant, the loess regression for combined age groups ($n = 75$) still clearly suggests a curvilinear relationship that is remarkably similar to the loess pattern found for Ricker's (1962) data scatter (Fig. 5).

ANOVA showed a significant ($P < 0.01$) difference in SAS among smolt size classes between 50 and 170 mm. The grouping with lower SAS (50–110 and >160 mm) overlapped the grouping with higher SAS (91–150 mm) at the 101–109 mm increment. Thus, SAS of smolts >100 mm were not statistically different.

Geographic Location (Latitude) and SAS

We grouped mean length and SAS of smolts from 19 sockeye nursery lakes into three latitude bands and three smolt size classes, i.e. a 3 × 3 factorial design (Table 2). Lake Washington in Seattle was the most southern (47° 30′N) system, while Larson Lake (61° 45′N) north of Anchorage was the most northern. We found no differences ($P = 0.21$) in smolt sizes associated with latitude, mean lengths within each size class being virtually identical across latitude bands (Table 3, matrix 1). SAS was significantly different due to both latitude and smolt size class, but there was no significant interaction between the two factors (two-way ANOVA) (Table 4). Small smolts in southern latitudes had SAS of 5.9% compared with a nearly threefold increase (17.7%) for an equally small smolt from higher latitude systems (Table 3, matrix 2). Similar SAS increases occurred for all smolt size groups as nursery lakes were located farther north. In addition, growing to a larger smolt

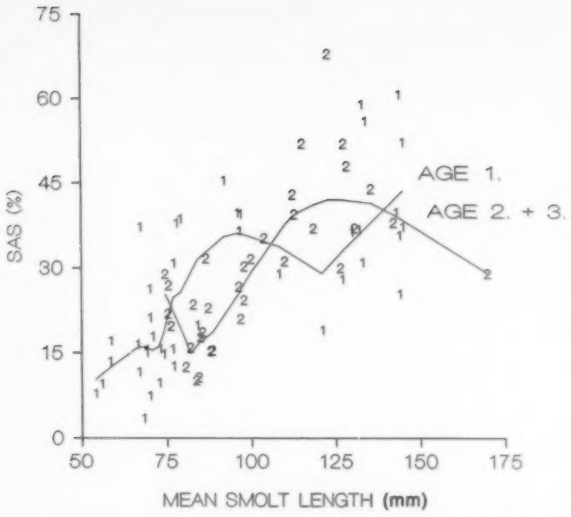


FIG. 4. Loess ($F = 0.4$) models relating SAS of age 1. and sockeye and age 2. + 3. smolts to mean lengths from 12 nursery systems located in Alaska with SAS of smolts >170 mm deleted.

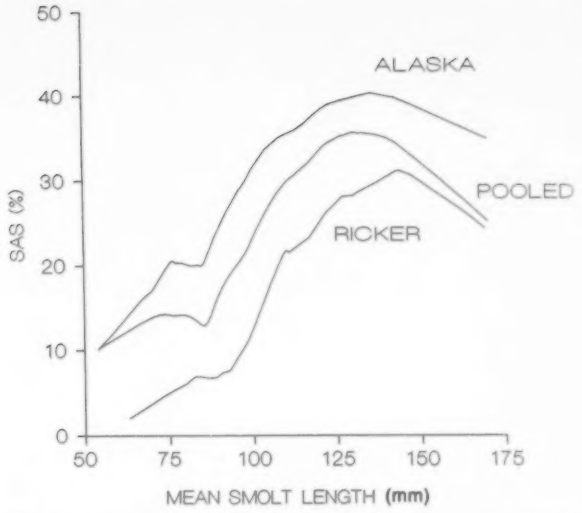


FIG. 5. Comparison among loess ($F = 0.4$) models relating SAS of sockeye to mean smolt lengths for six systems in Alaska, Canada, and Russia (Ricker 1962), 12 nursery systems in Alaska (Alaska), and all data and geographic locations (pooled).

TABLE 2. Sockeye nursery lake factorial matrix grouped by where the outlet enters the sea into three latitude bands and by smolt sizes into three ranges in smolt length. Sample size in parentheses. The decade when the data were taken follows the lake name.

	Latitude		
Smolt length	South (≤55°N)	Middle (>55°N, ≤60°N)	North (>60°N)
Small (>54 mm, <85 mm)	Cultus Chilko Babine (14)	Bare Leisure Hugh Smith McDonald (19)	Larson Packers Crescent Tustumena Eshamy (16)
Medium (≥85 mm, <115 mm)	Cultus Dalneye (15)	Karluk, 1930's McDonald Hugh Smith Bare Karluk, 1960's Leisure (12)	Packers Upper Russian Tustumena Eshamy (10)
Large (≥115 mm)	Washington Dalneye (15)	Karluk, 1930's Karluk, 1960's (22)	Hidden Big (14)

size within each of the latitude bands had survival advantages. In the southern band, growing larger than 85 mm doubled SAS to 12.6%, but growing still larger to >115 mm increased SAS by less than 50% to 17.1%. A sevenfold increase in SAS, from 5.9 to 39.1%, occurred while both growing larger and moving north, i.e. moving diagonally through the SAS matrix.

To further investigate the effects of geographic latitude of the nursery system on the smolt length – SAS relationship, we fit three separate loess models: one to each data set and a third to both data sets pooled (Fig. 5). Loess regression resulted in remarkably similar trends of higher SAS associated with larger smolt sizes. In fact, there appeared to be no difference in the underlying shape of any of these three relationships. Thus, we used the pooled data sets and fit three separate loess regressions using the latitude bands described in Table 2. Within the latitude bands, SAS tended to increase with increasing mean smolt length for all latitude bands: rho > 0.64; $P < 0.01$) (Fig. 6A–6C). Moreover, for the three latitude bands, increases in SAS were estimated to range between 0.3 and 0.5 (%) SAS·mm^{-1} across the smaller smolt sizes of <100 mm. Beyond about 110 mm, SAS appeared to plateau. Finally, SAS at any mean smolt length generally increased along a south to north latitude cline (Fig. 6D).

We next plotted the residuals from the pooled model in Fig. 5 and noticed that residuals from northern systems were generally greater than those from southern systems. We then fit loess models of SAS residuals as a function of mean smolt length for each latitude band (Fig. 7). For smolt sizes <100–110 mm, smolts from northern latitudes have higher SAS residuals (from the pooled model) than those from southern latitudes. Moreover, the relationship from middle latitude systems was between those of northern and southern latitudes. Above 110 mm, however, SAS residual models for the three latitude bands crossed, suggesting a lowered latitude effect at larger smolt sizes.

Finally, it is possible that age or time period (e.g. before or after 1976 (Mysak 1986)) also affected SAS. For example, because age 2. + 3. smolts may tend to be larger than age 1. smolts, effects of smolt length may have actually reflected differences between age classes. To examine this possibility, we analyzed SAS using latitude, age, and time period as factors with smolt length as a covariate. The sequential sums of squares in these analyses indicated that age was marginally significant ($P = 0.06$) and time period was highly significant ($P < 0.01$) with length already in the model. When latitude entered into the model, however, partial sums of squares showed that neither age ($P = 0.52$) nor time period ($P = 0.86$) contributed significantly to SAS. Moreover, median SAS before ($n = 23$) and after ($n = 14$) 1976 for Chilko Lake sockeye showed no difference ($P = 0.84$) even though median smolt lengths were the same ($P = 0.87$).

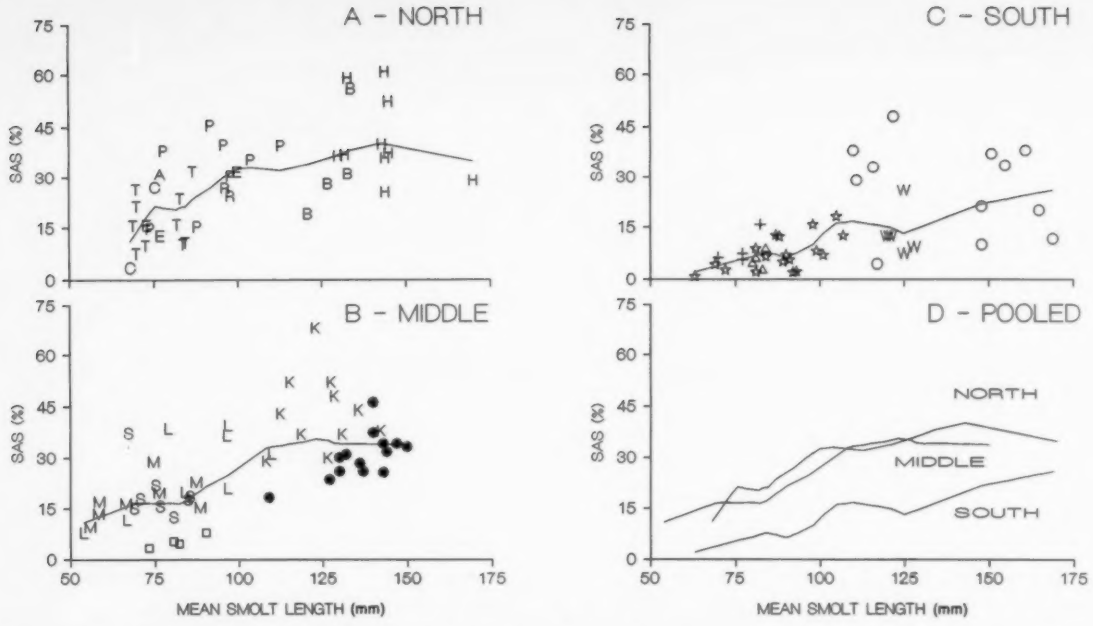


FIG. 6. Loess ($F = 0.4$) models relating SAS of sockeye to mean smolt lengths from nursery systems located within three broad geographic bands ((A) north, (B) middle, (C) south) as delineated in Table 2. Also shown is (D) the comparison among the three loess-latitude models without data points. Symbols identified as to nursery lake in Fig. 2 and letters identified in Fig. 3 with W identifying Lake Washington.

TABLE 3. Data matrices for mean sockeye smolt lengths (mm) and mean SAS (%) (with standard errors in parentheses) within three latitude bands and for three ranges in smolt length. Data correspond to lake and smolt size data in Table 2.

	Matrix 1: Length				Matrix 2: SAS		
Smolt length	South lat.	Middle lat.	North lat.	Smolt length	South lat.	Middle lat.	North lat.
Small	77.5 (6.5)	71.0 (9.2)	75.5 (5.6)	Small	5.9 (3.7)	16.7 (9.8)	17.7 (9.3)
Medium	97.2 (8.4)	97.2 (10.3)	97.2 (7.7)	Medium	12.6 (10.2)	25.1 (10.7)	32.0 (8.7)
Large	138.1 (19.1)	134.0 (9.2)	138.9 (11.8)	Large	17.1 (12.5)	37.0 (10.9)	39.1 (13.1)

SAS Patterns: Comparisons within Stocks

Cultus Lake

Foerster (1954) examined SAS (also included in the Ricker (1962) data set) for age 1. Cultus Lake smolts ($n = 18$) for 1927–44 and found a significant positive relationshp between smolt length (range 63–107 mm) and log SAS ($r^2 = 0.42$). The mean SAS of Cultus sockeye was 8.3%, while mean smolt size was 88.3 mm. We found that SAS (range 0.93–20.2%) increased with larger smolt sizes (rho = 0.49; $P < 0.05$). Using parametric linear regression, smolt length explained 38% of the variation in SAS which increased at a rate of 0.3 (%) SAS·mm^{-1} (Fig. 8). Thus, below 110 mm in length, increases in smolt size resulted in larger SAS, i.e. a positive, linear relationship.

Karluk Lake

In the 30 yr between smolt studies at Karluk Lake, smolt size dropped from a median of 138.5 mm to 127.2 mm ($P < 0.05$), but SAS increased ($P < 0.01$) from a median of 30.6% to 43.5% for the combined age groups. There was a negative (rho = −0.29) but nonsignificant ($P > 0.05$) relationship between smolt size and SAS. However, the negative rho value was consistent with the loess regression which also indicated that SAS dropped with an increase in smolt size (Fig. 9).

Separating SAS from both studies into age 2. and 3. groupings resulted in two significant, negative relationships (for each age group, rho values had $P < 0.05$) between smolt size and SAS (Fig. 9). Smolt size explained 42% ($P = 0.03$) of the variation in SAS for age 2. smolts (−0.8 (%) SAS·mm^{-1}) and

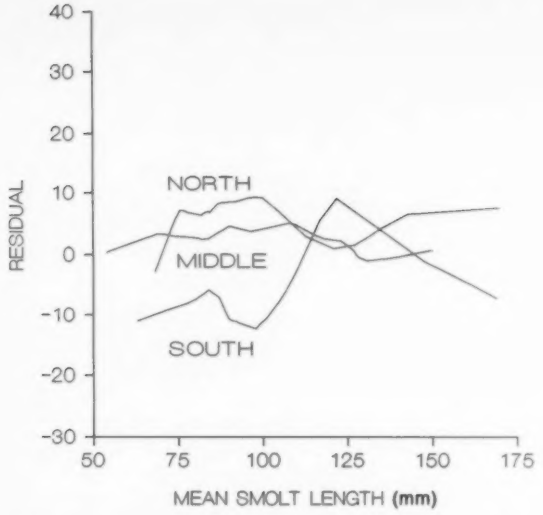


FIG. 7. Loess ($F = 0.4$) models fit to SAS residuals, from the loess SAS — smolt length relationship of pooled data sets (Fig. 5), as a function of mean smolt length for three latitude bands (north, middle, south) as defined in Table 2.

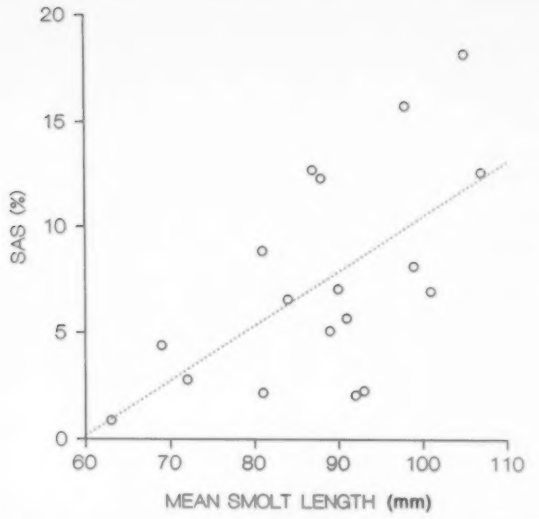


FIG. 8. Linear model relating SAS to mean smolt lengths for Cultus Lake sockeye.

66% ($P < 0.01$) of the variation in SAS for age 3. smolts (-1.2 (%) SAS·mm^{-1}). Thus, above 110 mm in length, increases in smolt size resulted in smaller SAS, i.e. negative, linear relationships.

The slopes of the age 2. and 3. regression (Fig. 9) were not significantly different (one-way ANCOVA; $P = 0.39$), suggesting a common mechanism responsible for changes in SAS, but older smolts had higher SAS ($P < 0.01$). The median lengths (128 and 140 mm) of the age 2. and 3. smolts, respectively, were different (Mann–Whitney U-test; $P < 0.05$). Thus, the older smolts were larger and survived better at sea.

Kvichak River

The range in smolt sizes from Alaskan sockeye stocks managed under a fixed escapement goal policy is expected to be small. Mean smolt lengths for Kvichak River (Bristol Bay, Alaska) sockeye generally followed this trend and centered around 80–90 mm. There was no significant relationship between smolt size and SAS, yet factors other than length may influence SAS. Such an effect is suggested by the increasing SAS of age 1. smolts relative to the percentage of comigrating age 2. smolts (Fig. 10). For the smolt years 1971–87 (1985 is excluded because of suspected undercounting of smolts), the percentage of age 2. smolts ranged from 3 to 99% and the SAS of age 1. smolts (mean size of 87.2 mm) ranged between 0.52 and 20.9%. Larger percentages of age 2. smolts increased the SAS for age 1. smolts (rho = 0.68; $P < 0.01$) explained 51% of the variation ($P < 0.01$) in age 1. SAS.

Also, increasing numbers of age 2. smolts increased the SAS for age 1. smolts (rho = 0.46; $P < 0.05$) and explained 36% of the variation ($P = 0.02$) in age 1. SAS. Finally, the median lengths ($n = 15$) of Kvichak River age 1. (87 mm) and age 2. (107 mm) smolts were different (Mann–Whitney U-test; $P < 0.01$), but the median SAS (9.3 versus 11.2%) were not ($P = 0.40$). Thus, the 20 mm larger size of the age 2. smolts over the age 1. comigrants did not infer an apparent SAS advantage.

TABLE 4. Summary of statistical significance levels (P) and percent of variation explained (%) by latitude and mean smolt length from a two-way ANOVA of 3 × 3 factorial matrices examining the ocean survival of sockeye.

	Matrix 1: Length		Matrix 2: SAS	
Factor	%	P	%	P
Latitude	<1	0.21	21	<0.01
Length	85	<0.01	30	<0.01
Length × latitude	<1	0.79	1	0.64

Discussion

Exploratory Approach

Ricker's (1962) graphical analysis of SAS has been reproduced in reviews of sockeye biology by Foerster (1968) and Burgner (1991). Within the data scatter are sockeye SAS from stocks that cluster in regard to smolt age, length, and geographical location. The latter two, relating SAS to smolt length and then to south to north latitude, appear to be of major importance whereas the third relating SAS to smolt age is less clear. These effects question the tacit assumption of length-dependent SAS over the entire smolt size range and argue for exploratory analyses using tools not available to Ricker (1962).

Latitude-Dependent SAS

Our analysis links increasing geographic latitude (Fig. 6D and 7; Table 3) with larger SAS, irrespective of smolt size, i.e. a south to north cline of increasing SAS. The existence of latitude as a cofactor of SAS explains differences in SAS for nursery systems producing similarly sized smolts. The mean length of smolts was independent of latitude, but SAS were clearly dependent on both latitude (21%) and length (30%) (Table 4). The effects of both factors on SAS resulted in Ricker's (1962) scatterplot (Fig. 1 and 2), as changes in SAS can now be tied to crossing latitude bands (e.g. Table 3, matrix 2) as well as to increasing smolt sizes. All SAS in the south latitude band below

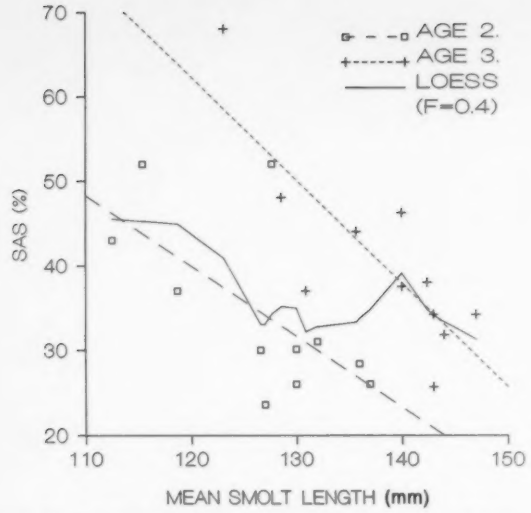


FIG. 9. SAS of Karluk Lake sockeye related to mean smolt lengths. Loess (F = 0.4) model fit to all data and separate linear models for age 2. and 3. smolts.

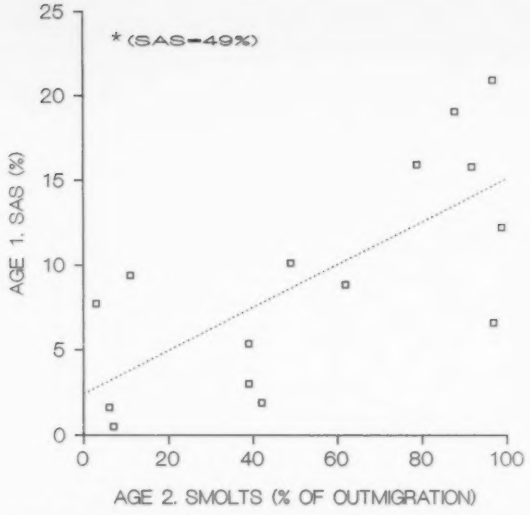


FIG. 10. SAS of Kvichak River sockeye in relation to the concurrent proportion of age 2. smolts in the outmigration. Data point denoted an asterisk was from 1985 and was not used in the linear model.

a smolt size of about 107 mm (Fig. 6*C*) are from Ricker's (1962) SAS plot. Yet, the SAS above 107 mm in Ricker's (1962) scatterplot are now split between the higher SAS of the midlatitudes and the lower SAS of southern latitudes (Fig. 6).

The variation in SAS between the Alaskan and Ricker (1962) data sets is based on changes in the latitude of where the smolts enter the sea. Loess analysis resulted in SAS estimates for 60-mm smolts from Alaska of 13% compared with 3% for 65 mm smolts in Ricker's (1962) data (Fig. 5). Also, smaller smolts in the northern latitude band had mean SAS of 18% compared with 6% in the southern latitude band (Table 3, matrix 2). These results reinforce existing sockeye SAS biostandards used in Alaska (12%) and Canada (4.7%) and may reflect conspecific differences in SAS along a latitude cline for threshold-sized smolts of about 60–65 mm. This threshold size (Geiger and Koenings 1991) has a physiological basis as the minimal size of seawater-adaptable sockeye smolts for freshwater rearing stocks (Koenings and Burkett 1987; L. B. Klyashtorin, VNIRO, Moscow, Russia, pers. comm.). As surmised by Bilton et al. (1982), SAS patterns for Pacific salmon may be fairly specific with reference to "gross geographic, climate, and ecological differences (e.g. Alaska vs. California)."

Length-Dependent SAS

Three levels of increasing resolution are consistent with the hypothesis that larger smolts have higher SAS. First, Ricker (1962) sought to explain broad changes in SAS using a large smolt–length range and several sockeye stocks. Using these data, Hyatt and Stockner (1985) fit a single SAS function across small and large smolt sizes. Later SAS from Alaskan stocks, however, only supported size-dependent SAS for smaller smolts (Koenings and Burkett 1987). Here, only SAS for the lower range of smolt sizes (<100 mm) increased (from loess regressions) at rates ranging from 0.3 to 0.5 (%) SAS·mm^{-1} within the three latitude bands (Fig. 6D). Second, SAS estimates for specific stocks, i.e. Cultus and Karluk Lake sockeye, each showed a distinct (although opposite) linear relationship to smolt size. As Cultus Lake smolt sizes were <110 mm and Karluk Lake smolts were >110 mm in size, loess analysis on a pooled SAS – smolt size data scatter would exhibit an arc-shaped pattern. Here, and perhaps for the larger data sets as well (Fig. 5), this characteristic pattern results from linking the functionally different SAS – smolt size relationships that exist for smolts smaller and larger than about 100 mm. Finally, Henderson and Cass (1991) suggested a linear size-dependent SAS relationship within a smolt (<90 mm) brood year for Chilko Lake sockeye and a decreasing linear fit for each of two other brood years because of lower SAS at larger smolt sizes.

These data argue for size-dependent SAS, but not as a continuous linear function across all smolt lengths, particularly if the data set includes smolts both above and below about 100 mm. When Alaskan sockeye SAS were compared using 10-mm increments (50–170 mm), movement from the lower SAS tier to the highest occurred at smolt sizes above 100 mm or 8 g. These statistical groupings compare favorably with (1) the plateau in SAS indicated by loess regression for the age 1. Alaskan smolts (Fig. 4) beyond 90 mm, (2) the flattening slopes of the loess regressions for SAS in the three latitude bands (Fig. 6D) beginning above smolt sizes of about 100–110 mm, and (3) coalescing SAS residual values at smolt sizes of about 110 mm. Historically, Ricker (1962) cited a visual "breakpoint" in SAS at 107 mm, and Peterman (1981) estimated that sockeye smolts of 110 mm have the least variable and highest SAS. Simply put, beyond a certain smolt length of about 100 mm, any further investment of energy into producing larger smolts results in diminishing increases or even decreases in SAS. A biological basis for these functionally distinct SAS zones may be found in the findings of Hartt (1980) and J. Orsi (pers. comm.) which suggest a habitat segregation of smolts <100 mm (nearshore) from smolts >100 mm (offshore).

Smolt Age-Dependent SAS

Overall, our analysis of the SAS of the 12 stocks within the Alaskan data set and the 18 stocks within the combined data

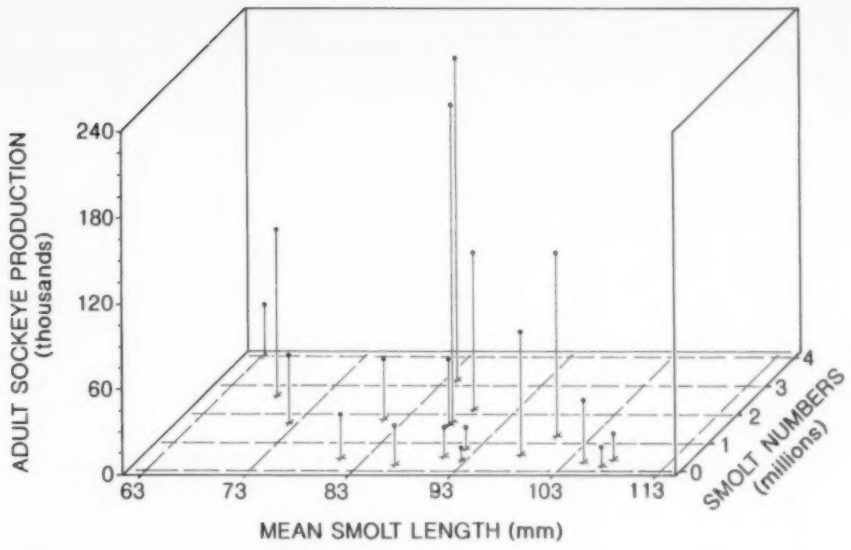


FIG. 11. Three-dimensional plot relating both mean smolt numbers and smolt sizes to resultant numbers of adult sockeye estimated to have returned to Cultus Lake.

sets found that smolt age was marginally significant compared with smolt length in explaining variation in SAS. As older smolts tended to be from more northern latitude nursery lakes, when latitude was included in the analysis, smolt age was no longer significant.

Yet, smolt age can impact SAS in part because of the influence of age on emigration timing, i.e. older smolts enter the sea first. A comparison of Alaskan SAS using loess regression found that age 2. smolts had lower SAS than comparably sized age 1. smolts until both reached about 110 mm (Fig. 4). Simply to survive at a rate equal to age 1. smolts, age 2. smolts needed to be about 20 mm longer. A larger size may compensate for heavier losses to predators given seasonally earlier entry into the sea. Larger numbers of age 2. smolts may even result in greater SAS of comigrating age 1. smolts as found for the Kvichak River (Fig. 10).

Further, the admix of smolts of different ages, even within a system, may complicate or even obscure SAS relationships. For example, the combined SAS for the large (> 110 mm) Karluk Lake age 2. and 3. smolts had no relationship to smolt size, although loess regression did suggest a negative, linear trend (Fig. 9). In contrast, relating SAS to smolt length by age classes revealed two significant, linear correlations. Establishing the age-specific relationships also allowed the determination (ANCOVA) that SAS was higher for age 3. smolts whereas a comparison of median SAS suggested that they were not.

Finally, in broad across-system comparisons, smolt sizes and latitude heavily influence SAS to the extent of excluding any impact of smolt age. Within a system, however, the earlier emigration of older smolts may reduce SAS below that expected from size alone. Whatever the cause, it is quite clear that older smolts do not necessarily survive better at sea simply because they are larger.

Smolt Size, SAS, and Adult Production

For the pooled data set (Table 2), the amount of variation in SAS above that associated with smolt length and latitude is nearly 50%. Other factors such as either local marine conditions (e.g. turbidity or salinity) or biological interactions may replace smolt size as a dominant factor given a limited smolt size range within a latitude band. In Alaska, a fixed escapement goal policy for managing sockeye stocks leads to a limited range in smolt sizes due to density-dependent rearing. In the Kvichak system, the percentages of age 2. smolts significantly affect the SAS of comigrating age 1. smolts (Fig. 10). As age 2. smolts migrate first into the sea, waiting predators may be satiated by the age 2. smolts enhancing the SAS of age 1. smolts while reducing the size-specific SAS of larger age 2. smolts.

Simply increasing escapements and thus reducing age 1. smolt sizes (Kyle et al. 1988) may lower adult returns as SAS decrease below a smolt length of 90–100 mm at about 0.3–0.5 (%) SAS·mm^{-1} (Fig. 4, 6, and 8). Analogous concerns were expressed by Mcdonald et al (1987) following an 8-mm decrease in the length of Babine Lake smolts to <80 mm. Mcdonald et al. (1978) suggest setting fry density targets for Babine Lake that produce mean smolt sizes >80 mm in order to optimize SAS without compromising freshwater survival, i.e. smolt numbers. Adult sockeye production at Cultus Lake is consistent with Mcdonald et al. (1987), as smolt populations in the size range of 85–100 mm produced five out of the six highest adult returns (Fig. 11). With density-dependent rearing in Cultus Lake, resultant smolt numbers at sizes <85 mm were not sufficient to counter declining SAS, and SAS at sizes >100 mm were not sufficient to counter declining smolt numbers. Moreover, decreases in adult production similar to those at Cultus Lake were found for Leisure Lake sockeye at smolt sizes above and below about 90 mm (Koenings and Burkett 1987). Geiger and Koenings (1991) suggested using such smolt information to reduce the risk of setting overly large escapement goals that could result in excessive fry densities.

SAS Patterns: A Biological Framework

Henry (1961), Straty and Jaenicke (1980), Peterman (1981), and Mcdonald et al. (1987) stressed the importance of early

marine residence and growth to sockeye SAS. Hartman et al. (1967) found that sockeye smolt emigration is seasonally dependent (earlier at lower latitudes) and suggested that timing is synchronized to the seasonal pulse in the marine food supply. After leaving freshwater, smolts <100 mm may remain nearshore. The association of length to SAS would be related to mortality rates upon initial entry into the nearshore environment, and nearshore residence times of smolts would vary inversely with size as does SAS (Table 3).

Larger sockeye smolts (>100 mm) tend to emigrate directly offshore, bypassing the littoral zone. Larger body size allows more efficient capture of pelagic zooplankton (e.g. copepods and amphipods) which have highly developed escape responses (Pearcy et al. 1989; Koenings and Kyle 1991). Similar size-specific, offshore behavior of larger (6.5 g) chum salmon (*Oncorhynchus keta*) juveniles increased SAS three- to fourfold above those of smaller (1–2.2 g) juveniles that reared nearshore (Pearcy et al. 1989). Hartt (1980) showed that the coastal migration routes of sockeye smolts during early marine residence were in a south to north direction. Presumably a more extended coastal migration to northern waters would cause smolts from southern stocks to undergo greater mortality (Fig. 6).

Parker (1962) suggested that differences in time spent in the coastal environment (as well as smolt size) may underlie changes in SAS on a south to north cline. Indeed, the association of latitude to SAS would be related to either the time period a smolt remained within the nearshore as determined by latitude-compensated growth rates (Conover 1990) or the extent of early marine migration (Hartt 1980). Thus, SAS increases both at higher latitudes and at larger sizes.

With SAS changing at rates between 0.3 and 0.5%·mm^{-1}, the implications of latitude- and length-dependent SAS to both management and enhancement programs are enormous. The dependence of SAS on smolt size coupled with the density dependence of juvenile growth and survival in freshwater (Koenings and Burkett 1987; Kyle et al. 1988) and to the temporal stability of zooplankton populations once depleted by overgrazing (Koenings and Kyle 1991) argue against overly large escapements implicit to adaptive management strategies (Geiger and Koenings 1991).

Finally, the implications of smolt size and latitude on lowering SAS of sockeye salmon place southern stocks at a serious disadvantage relative to either stock maintenance or rebuilding. However, the relationship of a larger smolt size (90–100 mm) to major increases in SAS perhaps caused by nearshore/offshore residence may lead to workable restoration strategies. We now view the age 1. smolt size of 90–100 mm (6–8 g) as the minimal size necessary to maximize the benefits of size after entering the sea.

Acknowledgments

We thank the following individuals who contributed to both the ideas and data included within this paper. Smolt data were provided by Gary Kyle (Tustumena, Hidden, and Larson lakes), Tim McDaniel and Larry Peltz (Eshamy and Big lakes), Pat Marcuson (Packers Lake), Nick Dudiak (Leisure Lake), and Mike Haddix and Tim Zadina (Hugh Smith and McDonald lakes).

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Influence of Environmental Variation and Spawning Stock Levels on Recruitment of Ocean Shrimp (*Pandalus jordani*)

Robert W. Hannah

Oregon Department of Fish and Wildlife, Marine Region, Marine Science Drive, Bldg. 3, Newport OR 97365, USA

Hannah, R. W. 1993. Influence of environmental variation and spawning stock levels on recruitment of ocean shrimp (*Pandalus jordani*). Can. J. Fish. Aquat. Sci. 50: 612–622.

The influence of environmental factors on recruitment variability in ocean shrimp (*Pandalus jordani* Rathbun) from 1967 to 1988 was investigated using correlation and multiple regression. Low sea levels during larval release in April, indicative of a strong spring transition in coastal currents, were strongly correlated with increased recruitment. Interannual changes in catch distribution showed an interaction between shrimp abundance and distribution, with reduced catches accompanying a northerly catch distribution. A linear model incorporating April sea level explained 58% of the variation in log recruitment and also correctly indicated a serious year class failure in 1989. An index of spawning stock was compared with the residuals from the linear model based on April sea level, but no stock–recruitment relationship was found.

L'auteur a étudié, par corrélation et régression multiple, l'influence de facteurs environnementaux sur la variabilité du recrutement de la crevette nordique (*Pandalus jordani* Rathbun) de 1967 à 1988. Le faible niveau de la mer pendant la période de production larvaire d'avril, qui est l'indice d'une forte transition de printemps des courants côtiers, était fortement corrélé avec l'augmentation du recrutement. Les variations interannuelles de la distribution des prises indiquaient l'existence d'une interaction entre l'abondance et la distribution des crevettes, une réduction des prises correspondant à une distribution plus vers le nord. Un modèle linéaire tenant compte du niveau de la mer en avril a permis d'expliquer 58 % de la variation du log du recrutement et indiquait, de façon exacte, l'important fléchissement de la classe de 1989. La comparaison d'un indice du stock de géniteurs avec les résidus du modèle linéaire du niveau de la mer en avril n'a pas permis de déceler de relation entre le stock et le recrutement.

Received April 14, 1992
Accepted September 8, 1992
(JB470)

Reçu le 14 avril 1992
Accepté le 8 septembre 1992

Fishable populations of ocean shrimp (*Pandalus jordani* Rathbun) are found from Queen Charlotte sound in British Columbia to California (Dahlstrom 1970). The trawl fishery for this species has exhibited large fluctuations in total catch, ranging from over 39 000 t in 1978 to only 5300 t in 1984 (PSMFC 1990). Some of this variation in catch can be attributed to fluctuations in fishing effort. The most influential factor, however, is probably variation in survival from the early larval stages to the age of recruitment. Pandalid shrimp fisheries frequently exhibit large-scale fluctuations in catch (Balsiger 1981; Clark and Anthony 1981; Gaffney 1981). These fluctuations have been attributed to a combination of changes in environmental factors effecting recruitment and rapid fishery development in response to dominant year classes (Balsiger 1981). Recruitment in other pandalid stocks and in penaeid shrimp stocks has been shown to fluctuate widely in response to changes in the environment (Dow 1964; Garcia 1983; Apollonio et al. 1986; Penn and Caputi1986; and others).

Previous research suggests that environmental variation in the northeast Pacific Ocean influences recruitment in ocean shrimp. In laboratory studies, Rothlisberg (1975) found that survival of ocean shrimp to the early larval stages declined rapidly above 11°C and that temperature tolerance widened at later larval stages. Ocean shrimp spawn in the fall each year, with females carrying the developing eggs until the following spring (Dahlstrom 1970). Larval release occurs during the months of March and April. The larvae are planktonic and are found in surface waters in the early stages, but occupy progressively greater depths as they develop. Rothlisberg (1975) suggested an early critical period for ocean shrimp during which survival could be strongly influenced by sea temperature. Rothlisberg and Miller (1983) also demonstrated a significant positive correlation between June–August upwelling on the Oregon coast and larval survival estimated from commercial landings data. Given the increased temperature tolerance of later stage larvae, and their tendency to occur at greater depth, the June–August time period is later than would be expected for a critical period based solely on sea temperature.

The 1989 year class (year of larval release unless noted) of ocean shrimp was virtually a complete failure. Catches of age 1 shrimp in the commercial trawl fishery were extremely low during the entire 1990 season, especially south of the Columbia River (Hannah and Jones 1991a). The upwelling indices used by Rothlisberg and Miller (1983) predicted recruitment for this year class to be slightly below average. The failure of the upwelling index to identify such a significant recruitment failure suggested that the relationship between ocean shrimp recruitment and the ocean environment warranted further research.

Huyer et al. (1979) demonstrated that there is a rapid "spring transition" in oceanographic conditions on the continental shelf off of Oregon each year. Winter conditions are generally dominated by northward shelf currents at all depths, winds from the south, and downwelling of coastal water. Spring–summer

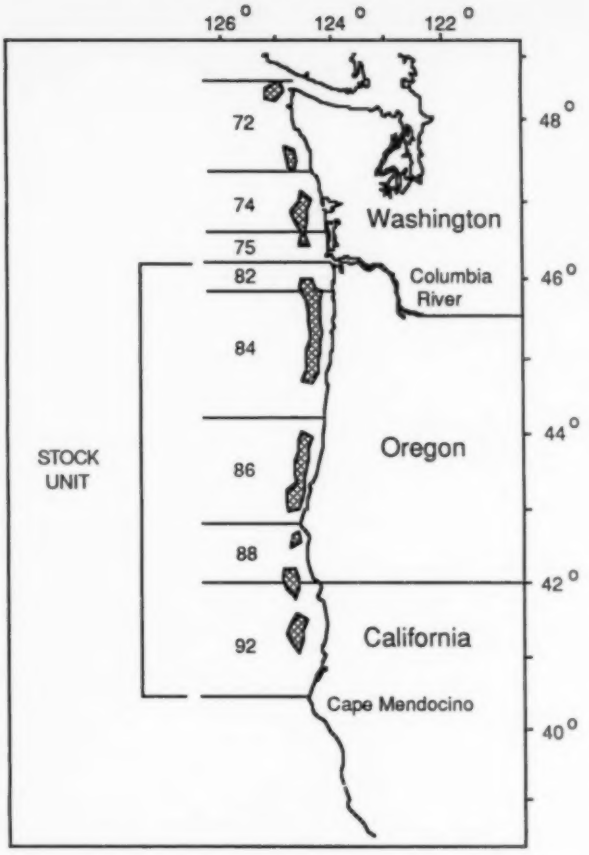

FIG. 1. Location of commercial concentrations of ocean shrimp along the U.S. Pacific coast (shaded areas) in PSMFC statistical areas 72–92.

conditions are characterized by strong southward surface currents, weak bottom currents, northwest winds, and coastal upwelling. The spring transition is most strongly reflected in coastal sea level measurements, which are generally elevated in the winter, fall rapidly in the spring, and remain depressed over the summer months (LaFond 1939; Patullo 1960; Huyer et al. 1979; Chelton et al. 1982; Hickey 1989). The timing of the spring transition coincides closely with ocean shrimp larval release. Coastal sea levels were lower than average in the winter and higher than average in the spring of 1989, suggesting a weak spring transition. The failure of the subsequent year class suggested that the intensity of the spring transition, as reflected in sea levels, may have an important influence on recruitment. The primary objective of this study was to test whether spring sea levels were correlated with ocean shrimp recruitment success.

Early attempts to develop a spawner–recruit relationship for ocean shrimp were unsuccessful (Abramson and Tomlinson 1972; Gotshall 1972). These studies, however, did not take into account the influence of variation in the environment as suggested by Garcia (1983) and others. A second objective of this study was to examine the evidence for a stock–recruitment relationship for ocean shrimp, after accounting for environmental effects.

Materials and Methods

The indices of recruitment and spawning stock used in this study were calculated from data on shrimp trawl activity for the years 1966–90. The study area extended from Cape Mendocino, California, to the Columbia River, Oregon (Fig. 1), including Pacific States Marine Fisheries Commission (PSMFC) statistical areas 82–92. The data used included total catch, fishing effort, and age and size composition of the catch for each area and month (Zirges et al. 1982; Oregon Department of Fish and Wildlife (ODFW), unpubl. data). Fishing effort was standardized for double-rigged and single-rigged trawl vessels (PFMC 1981) and expressed in single-rig equivalent hours (sre hours). For a further discussion of the data collection methods, see Hannah and Jones (1991b).

Age 2 shrimp were assumed to be fully recruited to shrimp trawl gear throughout the study area. Age 1 shrimp are incompletely recruited to the fishery (Lo 1978; ODFW, unpubl. data), especially early in the April–October fishing season. After 1978, the percentage of age 1 shrimp in the catch and mean size at age 1 increased (Hannah and Jones 1991b). A recruitment index based solely on the size of the population at age 2 would seriously underestimate recruitment after 1978. Therefore, two components were summed to calculate the index of shrimp recruitment, one based on the average catch-per-unit-effort (CPUE) for age 2 shrimp in April and May and one based on the catch of age 1 shrimp in the previous year. Each component of the index was calculated separately for each statistical area, summed across the five areas, and then combined.

The first component of the recruitment index, the average age 2 population for the April–May time period in year t, is estimated from April and May CPUE for age 2 shrimp, using the simplest form of Baranov's catch equation (Ricker 1975):

$$C/f = qN_0$$

where C = the number of age 2 shrimp caught in April and May, f = fishing effort (sre hours) in April and May, q = the catchability coefficient, and N_0 = the average age 2 population.

Prior to 1974, the fishing season for ocean shrimp in the study area opened 1 March. Afterwards, a 1 April opening date was standard. The April–May period was chosen to estimate the age 2 population because fishing effort and conditions are highly variable in March and early April, but substantial fishing effort develops by May. Using the simple sum of age 2 catch divided by the sum of effort is equivalent to an effort-weighted average of CPUE for the five areas. This approach to calculating CPUE was used to reduce the influence of increased variability in CPUE which can arise when one area receives a very low level of fishing effort. After the average age 2 population for the April–May period was calculated, the March and April catch of age 2 shrimp was added to the population estimate to generate the age 2 component of the recruitment index. This was necessary to standardize the estimates and minimize the influence of highly variable fishing effort in March and April. An estimate of q, the catchability coefficient, for area 92 was obtained from Geibel and Heimann (1976). It was assumed that q was constant at 0.0001017 and equal for each of the five areas.

The second component of the recruitment index is based on the catch of age 1 shrimp in year $t - 1$ and corrects for higher levels of harvest of age 1 shrimp after 1978. This component is calculated by discounting the monthly catch of age 1 shrimp from year $t - 1$ by an average monthly natural mortality rate

for the number of months that would have elapsed until 1 April of year t. The equation for the age 1 component is

$$N_1 = \sum_{i=1}^{n} C_i e^{-TM}$$

where N_1 = the age 1 component, C_i = the catch of age 1 shrimp in year $t - 1$ and month i, n = the number of months in the shrimp season in year $t - 1$, T = the number of months between month i and 1 April of year t, and M = the average instantaneous monthly rate of natural mortality.

The age 1 component actually represents a crude estimate of the number of additional age 2 shrimp that would have been present on 1 April of year t, if there had been no harvest of age 1 shrimp in year $t - 1$. The monthly rate of natural mortality used for discounting the age 1 catches was 0.096, obtained from Gotshall (1972). In employing this rate as a constant, it was assumed that there had been no time trends in natural mortality across the 1966–90 period. While this may be a reasonable assumption, monthly natural mortality rates are probably quite variable, and using a constant value injects an unknown level of additional variance into the recruitment index. Consequently, after 1976, when age 1 catch levels are higher, this index may be a less accurate measure of recruitment. Estimates of monthly or annual natural mortality rates are not available to correct for this problem.

A simple mean of the CPUE values (kilograms per sre hour) for all five areas, for the months of September and October, was used as an index of the ocean shrimp spawning stock. Since 1978, when shrimp size at 1 increased, age 1 shrimp have been fully recruited to trawl gear by September. In some of the early years of the fishery, especially when shrimp growth was poor, age 1 shrimp were probably not fully recruited to trawl gear by September (Lo 1978; ODFW, unpubl. data). Accordingly, the spawning stock index used in this study may understate the actual spawning biomass in some of the early years of the time series. However, this may still be the best index of the shrimp spawning stock available. Ocean shrimp are protrandric hermaphrodites. They have been shown to exhibit very flexible rates of sex change in response to fluctuations in age class structure (Charnov et al. 1978) and show evidence of density-dependent growth (Hannah and Jones 1991b). These findings suggest that the breeding population is likely to remain roughly balanced between males and females (Hannah and Jones 1991b) and show increased age-specific fecundity when growth is increased. Since data are not available to estimate annual age-specific fecundity, late-season biomass, if reflected accurately in changes in CPUE, is probably the best measure of egg production available.

One important difficulty in using CPUE as a proxy for abundance or biomass in ocean shrimp (i.e. assuming a constant q) is the effect of gear development and improvements in fleet efficiency from 1966 to the present (Hannah and Jones 1991b). The data on fishing effort employed in this study are corrected for the difference in efficiency between single-rigged and double-rigged vessels (one double-rigged hour is equivalent to 1.6 sre hours) but not explicitly for other changes in efficiency. These include the gradual increase in average vessel size over time, the switch in the late 1970's to high rise nets (Zirges and Robinson 1980; Hannah and Jones 1991b), and the development of sophisticated electronics for trawl vessels. There are several reasons why the bias introduced by these factors may be small enough to ignore. First, the time period during which the shrimp fleet was slowly converting from mostly single-rigged to mostly double-rigged vessels, 1974–80, is the same time period during which average vessel size increased, high rise nets became prevalent, and many of the advances in vessel electronics came into widespread use. In effect, the various improvements in fishing technology are confounded and may already be substantially accounted for in the single-rig to double-rig conversion factor. Second, there is evidence of gear interference at high effort levels (D. R. Bernard, Oregon State University, Corvallis, OR, 1983 draft), which would act to offset improvements in gear efficiency for many recent years. Gear interference in this fishery could arise from physical competition for space on the limited trawlable ground. A more likely source of interference could arise from the fleet spending more time fishing on less dense aggregations of shrimp than in the early years of the fishery, due to the stock being reduced from virgin levels. Finally, any bias that might be introduced to the recruitment index would be reduced after 1978 by the increased contribution of the age 1 component, which is not dependent on CPUE.

Although the primary objective of this study was to test whether spring sea levels were routinely related to recruitment success, a broad set of environmental measures from the pelagic larval time period were examined. The time period considered was limited to 10 mo after larval release, as it was assumed, a priori, that early larval or juvenile effects probably determined year class strength. The independent variables selected for analysis included sea level at Crescent City, California, sea surface temperature (SST) at 43°N, 125°W, and the coastal upwelling index at 45°N. The Crescent City sea level series was selected over other areas because it is close to the study area and has a very complete time series. For all of the variables, mean values from 2-mo time periods were used, beginning in March of the year of larval release and continuing through the following February. The environmental data were obtained from several sources. Sea level height data were provided by the National Oceanic and Atmospheric Administration (NOAA) archives, through the Sea and Lake Levels Branch. SST data originated from NOAA's Comprehensive Ocean-Atmosphere Data Set (COADS) described by Woodruff et al. (1987). Both the COADS temperatures and recent values for the upwelling index were obtained through the National Marine Fisheries Service's Pacific Marine Environmental Laboratory. The upwelling index for the years prior to 1973 came from Bakun (1973).

A stepwise process was used to analyze the relationship between the environmental variables and the shrimp recruitment index. First, scattergrams of each relationship were inspected for evidence of curvilinearity or heteroscedasticity. A number of the scattergrams indicated both effects, so the recruitment index was transformed using the natural logarithm (Ricker 1975). The evidence of log-linear relationships is not surprising, since minor changes in factors such as temperature or sea level can correspond to large-scale effects on the ocean environment. Correlation analysis was then used to assess the relationships between the environmental variables and the transformed recruitment index. The problem of serial autocorrelation within the individual time series was addressed using the method employed by Kope and Botsford (1990). This method results in an adjusted variance for the correlation coefficient, r, based on the autocorrelation functions of the two variables.

Initial results suggested that the 1982 data point was a clear

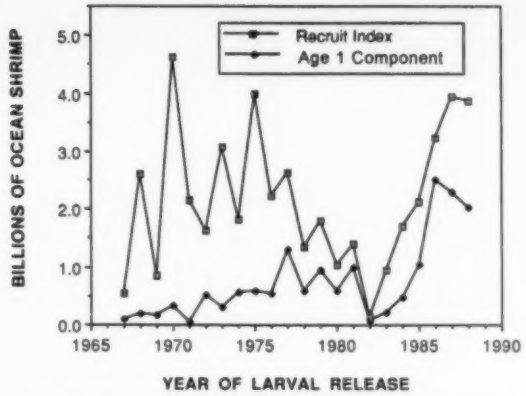

FIG. 2. Ocean shrimp recruitment index, and the age 1 component of the index, by year of larval release, 1967–88.

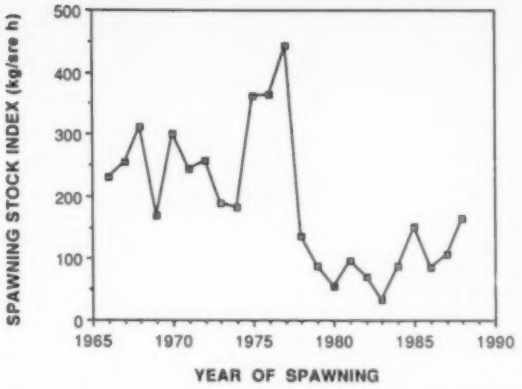

FIG. 3. Ocean shrimp spawning stock index (kg/single-rig equivalent h), 1966–88.

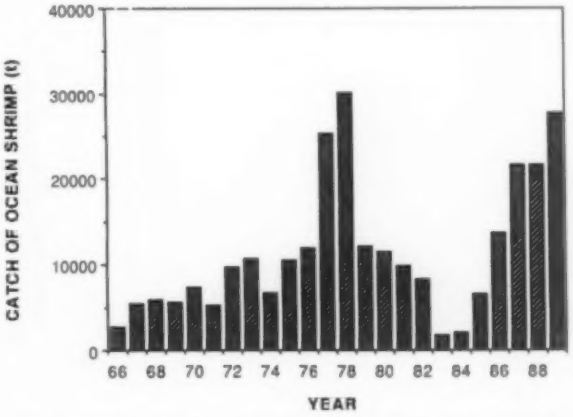

FIG. 4. Total catch of ocean shrimp from the study area, 1966–89.

outlier in many of the univariate correlations. Larvae released in 1982 developed during the 1982–83 El Niño event, which had extreme effects on fish stocks and zooplankton assemblages of the North Pacific Ocean (Miller et al. 1985; Pearcy et al. 1985). The primary interest of this investigation was in the more routine influence of the environment on shrimp recruitment, not the effect of extreme events. Accordingly, the univariate correlations were also calculated with the 1982 data point deleted. Next, linear regression was used to investigate, in more detail, the relationship between monthly mean sea level and $\log_e$ recruitment, for selected months. In this, and all subsequent analyses, the 1982 data point was excluded, and no correction for serial autocorrelation was used. Stepwise regression was then used to determine whether a combination of environmental factors could explain a major portion of the variation in the transformed recruitment index. Although no correction for serial autocorrelation was used in this analysis, variables were entered or removed from the stepwise regression at a significance level of $p < 0.01$ rather than $p < 0.05$ to prevent variables with weak explanatory power from entering the terminal model.

Walters and Collie (1988) have suggested that studies of fish–environment interactions have generally been more successful at demonstrating changes in distribution than changes in survival. There is abundant evidence that the environmental factors being examined here are closely tied with the strength of alongshore coastal currents (Reid and Mantyla 1976; Huyer and Smith 1985; Hickey 1989). Coastal currents are more coherent from surface to bottom in winter than in other seasons and could shift the geographical distribution of the adult population of shrimp, giving the appearance of an effect on recruitment (Huyer et al. 1979; Hickey 1989). Saelens and Zirges (1985) speculated that the 1982–83 El Niño event may have intensified the northward flowing Davidson current and shifted the entire stock northward for 1983. Although most work has focused on the onshore–offshore transport effects of upwelling, the magnitude of alongshore surface transport in the spring is likely to exceed the onshore–offshore component (Hickey 1989). To evaluate whether any apparent environmental effects on recruitment were really only influencing distribution, I also examined interannual changes in the percentage of the ocean shrimp catch taken inside the study area and from areas further north.

The residuals from the best environmental model were tested for evidence of a stock–recruitment relationship using a stepwise process. First, average values of the environmental variables were substituted into the model to calculate $\log_e$ recruitment under average environmental conditions. This value was then added to the model residuals and the resulting data series was back-transformed. This process provides a series of recruitment index values that are "adjusted" to average environmental conditions. The graph of these adjusted values versus the spawning stock index was inspected graphically for evidence of a stock–recruitment relationship and to determine the approximate form of the function. Garcia (1983) has suggested that many apparent stock–recruitment relationships in shrimp stocks are artifacts arising from serial autocorrelation within the recruitment indices of short-lived stocks. The adjusted recruitment index values were examined for evidence of correlation at a lag of 1 yr to address this concern. Multiple linear regression was then used to test the fit of a composite model incorporating the apparent stock–recruitment function.

Results

The ocean shrimp recruitment index (Fig. 2) shows wide fluctuations from 1967 to 1988 but no evidence of a sustained decline. The index of spawning stock biomass (Fig. 3) is generally decreased after 1977, due to some years of low recruitment

TABLE 1. Linear correlations between the natural logarithm of the ocean shrimp recruitment index and selected environmental variables. Coefficients significant at the 0.05 level using simple correlatin methods are shown. Asterisks depict levels of significance after correction for serial autocorrelation within a series (*$p < 0.05$; **$p < 0.01$).

Variable	Time period	Months after larval release	Correlation coefficient
Sea surface temperature	Mar.–Apr.	0	ns
	May–June	2	ns
	July–Aug.	4	ns
	Sept.–Oct.	6	−0.490
	Nov.–Dec.	8	ns
	Jan.–Feb.	10	−0.537*
Upwelling index	Mar.–Apr.	0	ns
	May–June	2	−0.469*
	July–Aug.	4	ns
	Sept.–Oct.	6	0.439
	Nov.–Dec.	8	ns
	Jan.–Feb.	10	0.688**
Sea level height	Mar.–Apr.	0	−0.648**
	May–June	2	ns
	July–Aug.	4	ns
	Sept.–Oct.	6	−0.577*
	Nov.–Dec.	8	ns
	Jan.–Feb.	10	−0.597*

TABLE 2. Crosscorrelations of mean sea level (SL) at Crescent City, California, mean sea surface temperature (SST) at 43°N, 125°W, and the mean upwelling index (U) at 45°N, 125°W for selected 2-mo intervals for 1967–88. Asterisks depict standard levels of significance without correction for serial autocorrelation between years (*$p < 0.05$; **$p < 0.01$).

Variable	Months after larval release	Mar.–Apr. SL	Sept.–Oct. SL	Jan.–Feb. SL	Jan.–Feb. SST	May–June *U*	Jan.–Feb. U
Mar.–Apr. SL	0	1.000					
Sept.–Oct. SL	8	0.552**	1.000				
Jan.–Feb. SL	10	0.127	0.617**	1.000			
Jan.–Feb. SST	10	0.158	0.695**	0.642**	1.000		
May–June U	2	0.316	0.211	0.451*	0.138	1.000	
Jan.–Feb. U	10	−0.235	−0.496*	−0.793**	−0.738**	−0.420	1.000

and several years of high catch (Fig. 4). Although not shown, the graph of the recruitment index versus the parent year spawning biomass index showed no discernible relationship. This suggests that either environmental effects have been the primary influence on recruitment over the years in question or errors in the indices are obscuring any relationship.

The correlation analysis (Table 1) shows several significant linear correlations ($p < 0.05$) between the environmental variables tested and $\log_e$ of the recruitment index. Sea level and SST are consistently negatively correlated with shrimp recruitment. The upwelling index shows both positive and negative correlations with recruitment, suggesting a more complex relationship or spurious correlations. Six of the 18 comparisons are significant, even after correction for serial autocorrelation. Only one significant correlation in 20 would be expected by chance alone. The correction for serial autocorrelation reduced the significance level for several of the correlations. The high number of significant correlations is partially explained by crosscorrelation between the variables (Table 2). The upwelling index, sea level, and SST are well correlated in January–February. Many of the other variables show some degree of crosscorrelation as well. The six significant correlations clearly represent a much smaller number of real environmental effects on shrimp recruitment.

Scattergrams of several of the significant environmental variables (Fig. 5–8) show that for many of the variables, the 1982 data point is an outlier. The low recruitment to the study area for this year class may have resulted from the stock being shifted northward in the winter of 1983 (calendar year) rather than from effects on shrimp survival (Saelens and Zirges 1985). With 1982 excluded (Table 3), March–April sea level shows by far the strongest correlation with shrimp recruitment ($p < 0.01$). The variables related to winter ocean conditions 10 mo after larval release, which show good correlations with $\log_e$ shrimp recruitment in Table 1, all show reduced correlation coefficients in Table 3. May–June sea level and September–Otober SST and sea level are also correlated with recruitment success ($p < 0.05$), but less strongly than March–April

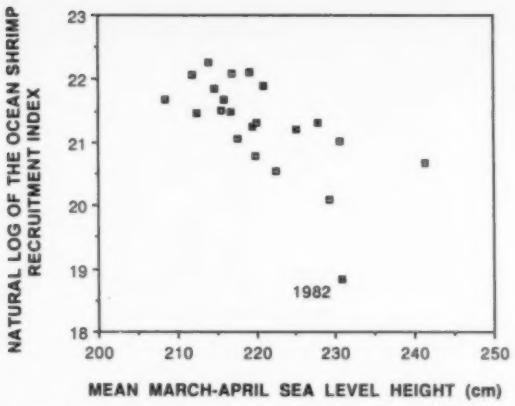

FIG. 5. Natural logarithm of the ocean shrimp recruitment index versus mean sea level at Crescent City, California, during March–April of the year of larval release. Data shown are for spring larval release, 1967–88.

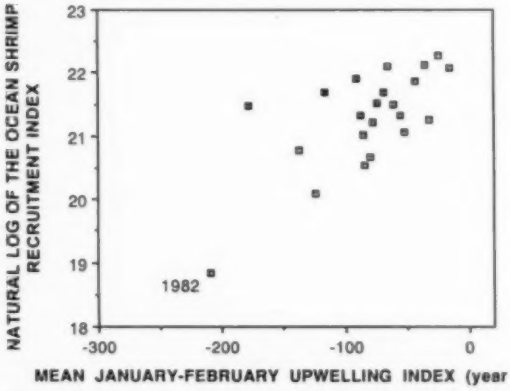

FIG. 6. Natural logarithm of the ocean shrimp recruitment index versus the mean upwelling index at 45°N, 125°W during January–February of the year following larval release. Data shown are for spring larval release, 1967–88.

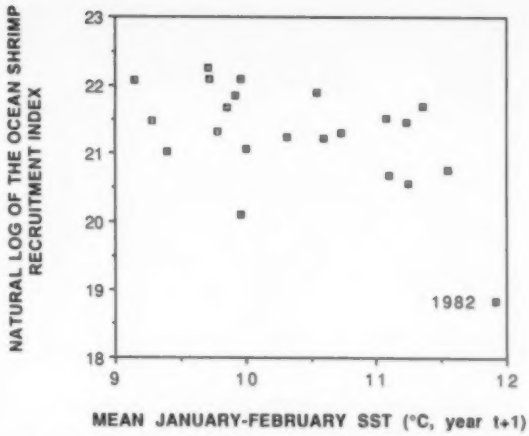

FIG. 7. Natural logarithm of the ocean shrimp recruitment index versus mean SST at 43°N,125°W during January–February of the year following larval release. Data shown are for spring larval release, 1967–88.

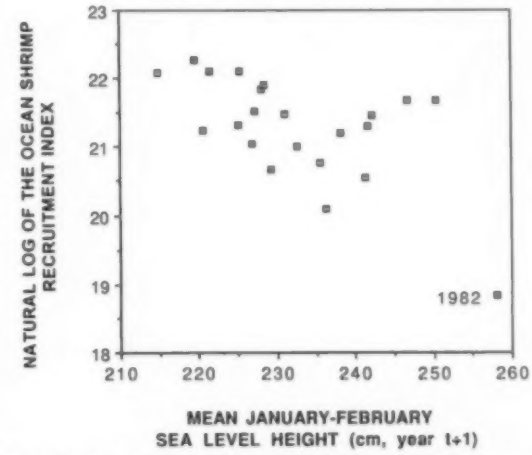

FIG. 8. Naural logarithm of the ocean shrimp recruitment index versus mean sea level at Crescent City, California, during January–February of the year following larval release. Data shown are for spring larval release, 1967–88.

sea level. Due to the data gap created by deleting the 1982 data point, the comparisons in Table 3 are not corrected for serial autocorrelation. This leaves some doubt as to whether some of the marginally significant correlations would remain so after such correction.

Linear regressions of $\log_e$ recruitment versus mean sea level for March and April show that April sea level is a better single predictor of shrimp recruitment than either March sea level or the March–April mean (Fig. 9 and 10; Table 3). April sea level alone explains 58% of the variation in $\log_e$ recruitment ($p < 0.005$), while the regression with March sea level is nonsignificant. Stepwise regression analysis on all of the variables results in a simple linear regression model based on April sea level, suggesting that the other environmental variables do not explain a significant degree of residual variation in shrimp recruitment ($p > 0.01$). Fitted values (back-transformed) from this model mirror most of the major fluctuations in the recruitment index from 1967 to 1988 (Fig. 11). The fact that the model incorporates March–April sea level with a negative sign, as was suspected a priori, is encouraging. The model also predicts a recruitment level of 1.3 billion shrimp for the failed 1989 year class. This would be well below average for the 21-yr time series and lie within the bottom 25% of observed recruitment values.

These data provide evidence that sea level at the time of larval release exerts a strong and routine effect on subsequent shrimp recruitment to the study area. One alternative hypothesis worth examining is that April sea level primarily influences shrimp distribution. The drop in spring sea level is associated with the intensification of southward surface flow (Huyer et al. 1979), which could simply carry more larvae into the study area. If such shifts in distribution are the dominant mechanism by which the shrimp population within the study area is determined, then we should see a tendency for high catches within the study area to be associated with reduced catches to the north. This effect should be especially strong when we compare catch

TABLE 3. Linear correlations between the natural logarithm of the ocean shrimp recruitment index and selected environmental variables, with the 1982 data point deleted. Coefficients significant at the 0.05 level using simple correlation methods are shown. Asterisks depict standard levels of significance without correction for serial autocorrelation (*$p < 0.05$; **$p < 0.01$).

Variable	Time period	Months after larval release	Correlation coefficient
Sea surface temperature	Mar.–Apr.	0	ns
	May–June	2	ns
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	Nov.–Dec.	8	ns
	Jan.–Feb.	10	ns
Upwelling index	Mar.–Apr.	0	ns
	May–June	2	ns
	July–Aug.	4	ns
	Sept.–Oct.	6	ns
	Nov.–Dec.	8	ns
	Jan.–Feb.	10	0.473*
Sea level height	Mar.–Apr.	0	−0.645**
	May–June	2	−0.471*
	July–Aug.	4	ns
	Sept.–Oct.	6	−0.497*
	Nov.–Dec.	8	ns
	Jan.–Feb.	10	ns

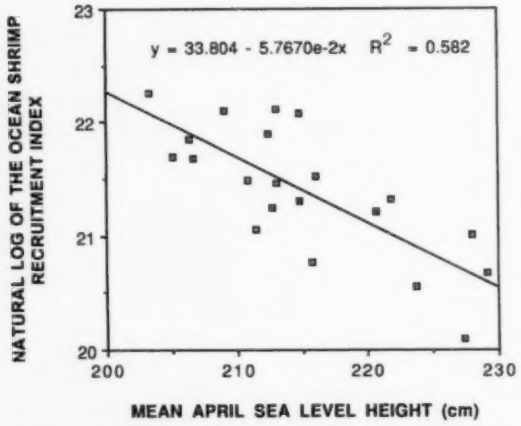

FIG. 9. Linear regression of the natural logarithm of the ocean shrimp recruitment index versus mean sea level at Crescent City, California, in April of the year of larval release. Data shown are for spring larval release, 1967–88, excluding 1982.

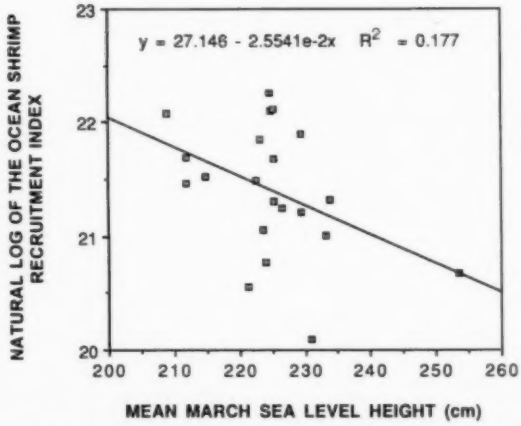

FIG. 10. Linear regression of the natural logarithm of the ocean shrimp recruitment index versus mean sea level at Crescent City, California, in March of the year of larval release. Data shown are for spring larval release, 1967–88, excluding 1982.

in the study area with catch in British Columbia, the northern limit of commercial concentrations of *P. jordani*.

The data on interannual changes in catch distribution suggest a link between effects on shrimp distribution and shrimp abundance. Table 4 shows the total catch of pandalid shrimp for the study area and from waters off Washington and British Columbia (PSMFC area 66 only) for 1979–89 (year of catch). Prior to 1979, the distribution of catch is probably not a good indicator of the distribution of recruits, since the catch was still dominated by age 2 and 3 shrimp (Hannah and Jones 1991b). Afterwards, age 1 shrimp dominated the catch from the study area. For these areas, the total pandalid catch is dominated by *P. jordani* (Butler 1980). Also shown is the percentage of the total catch from north of the Washington–Oregon border. This table provides evidence for a northward shift of the stock in 1983, but shows a much stronger tendency for catches to be positively correlated along the coast. Catch north of the Washington–Oregon border shows a significant positive correlation ($p < 0.01$) with both catch to the south and the shrimp recruitment index for the same catch year ($p < 0.05$). Catch from the study area is also positively correlated with catch from British Columbia ($p < 0.01$). The table also shows a tendency for large total catch to be associated with a more southerly catch distribution, and reduced catch with a northerly distribution, an

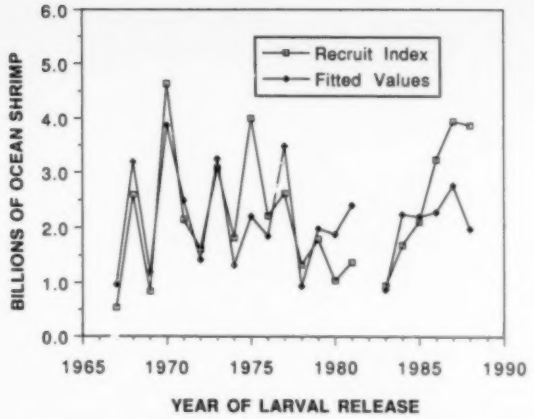

FIG. 11. Ocean shrimp recruitment index versus predicted values (back-transformed) from the environmental model based on April sea level for larval release years 1967–88.

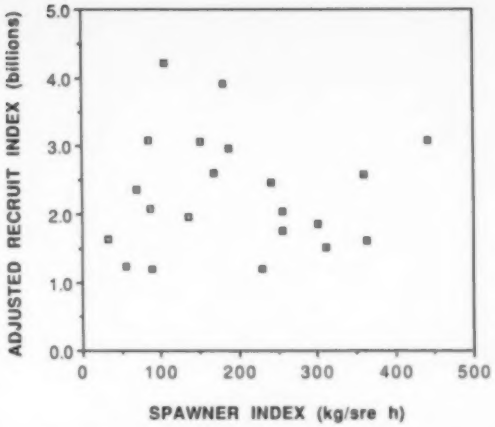

FIG. 12. Ocean shrimp recruitment index, adjusted for environmental effects, versus the spawning stock index from the parent year.

TABLE 4. Landings (t) of pandalid shrimp, primarily *P. jordani*, from the study area (Oregon and northern California) and from waters off of Washington and British Columbia[a], 1979–89. Percentage of total landings from north of the Washington–Oregon border is also shown.

Year	British Columbia	Washington	Study area	Total	Northern percentage
1979	235	8573	12104	20912	42.1
1980	136	9099	11467	20702	44.6
1981	403	7730	9923	18056	45.0
1982	183	4197	8231	12611	34.7
1983	40	3910	1702	5652	69.9
1984	41	2180	2144	4365	50.9
1985	382	5909	6843	12774	49.3
1986	1057	12056	13662	26775	49.0
1987	2535	8644	21718	32897	34.0
1988	2223	10161	21670	34054	36.4
1989	2169	7824	27738	37730	26.5

[a]British Columbia landings are from PSMFC area 66 only.

indication of interaction between factors influencing abundance and distribution of recruits.

A closer examination of the effects of the 1982–83 El Niño event on shrimp catch distribution suggests that winter ocean conditions may be having more of an effect on shrimp distribution, while spring sea levels are more directly influencing survival. This ENSO event caused a very strongly negative January–February upwelling index and elevated March–April sea levels in calendar year 1983, which should have influenced recruitment of the 1982 and 1983 year classes, respectively. The catch distribution for 1983 (1982 year class), presumably influenced by winter ocean conditions in 1983, shows a definite northward shift (Table 4), while the catch distribution for 1984 does not. However, catch in 1984 is even lower than in 1983. Of course, interannual changes in the distribution of catch could result from differential survival, rather than a wholesale shifting of the stock northward.

The recruitment index, after adjustment for the effects of April sea level, is shown versus the spawning stock index in Fig. 12. The scattergram shows, if anything, a vague suggestion of a domed (Ricker type) stock–recruitment relationship. The adjusted recruitment index shows no evidence of significant autocorrelation at a lag of 1 yr ($p > 0.05$), which would complicate testing for a statistically significant stock–recruitment relationship. The results of fitting a composite model incorporating the Ricker stock–recruitment relationship is shown in Table 5. The low partial F-values for the stock–recruitment terms suggest that changes in spawning stock do not explain a useful degree of the residual variation in shrimp recruitment after accounting for environmental variation.

Discussion

The analysis presented here shows that internannual variation in sea level at the time of larval release correlates well with variation in ocean shrimp recruitment. This connection between recruitment in ocean shrimp and the ocean environment is not a new idea. However, these results suggest different mechanisms by which the environment may be influencing recruitment. It has been argued by Rothlisberg and Miller (1983) that surface water temperatures during the larval phase may directly impact survival. Rothlisberg (1975) demonstrated that temperature has a strong influence on survival of ocean shrimp larvae in the laboratory. This study showed that the spring drop in sea level influences recruitment but did not identify spring or early summer SST as important. Early spring

TABLE 5. Results of fitting a composite model incorporating the Ricker stock–recruitment function, using multiple linear regression. Data are for larval release years 1967–88, excluding 1982. Model is $\log_e$(recruit index) = $a + b_1(\log_e$(spawner index)) + b_2(spawner index) + b_3(April sea level). ***$p <$ 0.005.

Parameter	Coefficient	95% confidence interval		Partial F-value	R^2 (adjusted)
		Lower	Upper		
a	31.745				
b_1	0.426	−0.0418	1.271	1.133 ns	
b_2	−0.002	−0.007	0.003	0.899 ns	
b_3	−0.056	−0.031	−0.076	23.059***	
Full model					0.537

SST's are generally within the range of 8–11°C found to be favorable for early larval survival (Rothlisberg 1975; Hickey 1989) and are higher, and consequently more critical to larvae, later in the year. Since larvae occupy greater depths as they develop and show a wider temperature tolerance as well, it is unlikely that water temperature directly limits shrimp survival except in very warm years, or in combination with reduced salinity (Rothlisberg 1975).

Several authors have argued that very strong spring upwelling may be detrimental to recruitment in species with planktonic larval forms by causing excessive offshore transport of larvae (Parrish et al. 1981). This study provides very little evidence to support this hypothesis for ocean shrimp. Figure 9 shows no evidence that $\log_e$ recruitment declines at very low spring sea levels, which would be evidence of an adverse impact caused by an excessively strong spring transition. The existence of subsurface onshore return flows (Hickey 1989) and the fact that larvae occupy deeper water as they develop argue that onshore–offshore larval transport may be quite complex.

It has also been suggested that the onset of spring upwelling transports cold, nutrient-rich water to the surface, favoring the development of larval food sources (Rothlisberg and Miller 1983). The results of this study are consistent with this hypothesis, even though the March–April upwelling index does not correlate directly with recruitment. The work of Huyer et al. (1979), describing the spring transition in detail for 1973 and 1975, suggests that the spring transition is triggered by southward wind of sufficient duration to produce a critical level of cumulative offshore Ekman transport. It follows that the drop in sea level in March–April is a more direct indicator of the onset of the upwelling season than the mean March–April upwelling index. The upwelling index is an estimate of wind-driven offshore Ekman transport which, on a mean monthly basis, may not reliably indicate whether an event of critical intensity and duration has occurred.

The data on interannual changes in the north–south distribution of catch suggest that changes in ocean shrimp abundance are positively correlated over a wide area of the Pacific coast. Northerly shifts in catch distribution also coincide with low total stock size, while increased stock size is associated with a more southerly distribution, suggesting a complex interaction between distribution and abundance. Although previous hypotheses have emphasized the importance of nutrients, this interaction suggests another hypothesis for how the timing of the spring transition could be influencing shrimp larval survival. With an early spring transition, initial larval transport should be southward and offshore and northward and onshore for a delayed transition. The onshore transport that precedes a late spring transition could carry larvae into very nearshore waters and into the surf zone, where poor survival would be expected. The fact that weak year classes fair better in the northern part of the stock range could be because the shrimp grounds are farther offshore, due to a wider continental shelf (Hickey 1989). Accordingly, it would take a longer period of onshore transport to have the same detrimental effect on recruitment in the northern area. There are, of course, other differences in the ocean environment north and south of the Washington–Oregon border, such as temperature and the seasonal influence of the Columbia River plume, which could also be causing the observed differences in how a late spring transition affects recruitment. A thorough study of how environmental factors correlate with shrimp recruitment success off the Washington coast may help explain this interaction between shrimp catch distribution and recruitment success.

This study provides virtually no evidence for a meaningful stock–recruitment relationship for ocean shrimp. This is not surprising, since stock–recruitment functions are very difficult to detect in stocks with high and variable rates of natural mortality (Koslow 1992) and in stocks with environmentally driven recruitment (Goodyear and Christensen 1984). Penn and Caputi (1986) fit a Ricker-type curve to spawning stock and recruitment data for the tiger prawn (*Penaeus esculentes*) but presented little evidence in support of the selection of any particular recruitment function. It has been assumed that, for penaeid shrimp stocks, the asymptotic curve of Beverton and Holt (1957) is appropriate (Neal 1975; Garcia 1983). This assumption is based on the concept that estuarine nursery areas utilized by penaeids may have a relatively constant carrying capacity. This line of reasoning is invalid for ocean shrimp which do not utilize estuaries. The most appropriate form of recruitment curves for pandalid shrimp remains an open question.

The hazards of correlative studies of recruitment and environmental effects have been discussed thoroughly by a variety of authors (Shepherd et al. 1984; Sissenwine 1984; Walters 1985; Walters and Collie 1988; and others). This study corrects for serial autocorrelation and attempts to adjust for some of the other pitfalls of exploratory correlation analysis. Even so, the residuals from the best environmental model are sufficiently large as to render it of limited utility for predicting shrimp recruitment. Even if further development does yield an adequate prediction equation for shrimp recruitment, it is difficult to see how this would be useful for management of the stock (Walters and Collie 1988).

Correlative studies do serve useful purposes, however, and may be especially important for shrimp stocks. It is generally accepted that recruitment in shrimp stocks is primarily envi-

ronmentally driven, while stock–recruitment relationships have only rarely been demonstrated (Garcia 1983). Research on environmental factors can help to develop hypotheses about the mechanisms by which the environment determines recruitment levels, which can then be further tested using more specific approaches. Ocean shrimp, in particular, exhibit a life history that seems resistant to over-harvest (Hannah and Jones 1991b). The fact that environmental factors have substantial influence in determining recruitment success further argues that fishing is not depressing recruitment. However, to ignore the potential effect of fishing on parent stock levels defies the common sense notion that some underlying relationship between parent stock and recruitment must exist. Shepherd (1982) has stressed that the most fundamental aspect of a stock–recruitment relationship is that zero parent stock provides for zero recruitment and that fisheries do collapse. This study supports the contention of Garcia (1983), and others, that a thorough understanding of the environmental mechanisms which influence shrimp recruitment must be gained before meaningful information on how fishing influences recruitment will emerge.

Acknowledgements

This project was financed in part with Federal Interjurisdictional Fisheries Act funds through the U.S. National Marine Fisheries Service. Several individuals provided assistance in the completion of this project. S. A. Jones, J. Golden, and P. Lawson provided many constructive comments on the form and content of the manuscript. Helpful reviews of early drafts were provided by L. Botsford of the University of California, Davis, and A. Huyer of Oregon State University.

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Estimating Crayfish Density in Lakes Using Quadrats: Maximizing Precision and Efficiency[1]

S. Lamontagne[2] and J. B. Rasmussen

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1, Canada

Lamontagne, S., and J. B. Rasmussen. 1993. Estimating crayfish density in lakes using quadrats: maximizing precision and efficiency. Can. J. Fish. Aquat. Sci. 50: 623–626.

We provide a method to estimate the number of quadrat samples needed to obtain precise crayfish densities at a site or transect for two quadrat sizes (1 and 10 m^2). We based the analysis on data from a survey of 61 depth-transects from 31 lakes representing a gradient in crayfish density. There was no significant difference between density estimates obtained with different quadrat sizes for six transects where both quadrats were used. Fewer samples were needed with the large quadrat, but it was more efficient to use a 1-m^2 quadrat at densities above 0.3 crayfish·m^{-2} because less effort (i.e. diving time) was required to collect the requisite number of samples. There was a linear relationship between density estimates by quadrat and timed counts (a more rapid method to estimate crayfish density), but timed counts were less accurate.

Nous présentons une méthode pour estimer le nombre d'échantillon par quadrat nécessaire pour une mesure précise de la densité de l'écrevisse, pour un site ou transect, pour deux grandeurs de quadrat (1 et 10 m^2). Nous basons notre analyse sur des données recueillies de 61 transects de 31 lacs. Il n'y avait pas de différence significative entre les densités obtenues avec les différentes grandeurs de quadrat pour six transects ou les deux quadrats ont été utilisé. Moins d'échantillons étaient nécessaire avec le grand quadrat. Cependant, il était plus efficace d'utiliser le quadrat de 1 m^2 pour les densités au-dessus de 0,3 écrevisse·m^{-2} parce que moins d'effort (i.e. temps de plongée) était nécessaire pour recueillir le nombre désiré d'échantillon. Il y avait une relation linéaire entre les estimés obtenus par quadrat et ceux obtenus par recherche chronométrée (une méthode plus rapide pour obtenir un estimé de densité), mais la recherche chronométrée est moins exacte.

Received October 30, 1991
Accepted September 8, 1992
(JB287)

Reçu le 30 octobre 1991
Accepté le 8 septembre 1992

SCUBA sampling is the most accurate method to obtain information about crayfish populations in lakes (Malley and Reynolds 1979; Davies and Ramsey 1989; France et al. 1991). Underwater sampling is practical because in most lakes, crayfish are restricted to the shallow waters of the littoral zone (Capelli and Magnuson 1976). Crayfish can be collected at night when they are exposed on the substrate (Davies and Ramsey 1989), or by turning logs, boulders, and other shelters they use during the day. Quadrats, belt-transects, and timed counts have been used to obtain abundance estimates with SCUBA (Abrahamsson and Goldman 1970; Capelli and Magnuson 1976; Stein 1977; Collins et al. 1983; Davies 1989; Quinn and Janssen 1989). Because a limited time can be spent SCUBA sampling, a sampling program of crayfish populations should maximize the precision of crayfish abundance estimates with the least effort.

In this study we compare the precision and efficiency of 1- and 10-m^2 quadrats to sample crayfish populations. We first established a relationship between mean density and sampling variance ($\bar{X}$:s^2) for crayfish from the data collected during a survey of 61 depth-transects from 31 lakes. Using this relationship, we determined for each quadrat size the number of replicate samples needed at different densities to achieve various levels of precision. We then demonstrate that along a gradient in crayfish density, a threshold exists where one should switch from one quadrat size to the other to maximize sampling efficiency. Also, we compare density estimates obtained by quadrats with those obtained from timed counts (Collins et al. 1983), a more rapid method to estimate population size.

Methods

Data Collection

We collected crayfish from 31 lakes in Quebec, Ontario, and New York State in the spring and summer of 1989 and 1990 (data set available from the authors upon request). A large gradient in crayfish density was sampled with both quadrat sizes. The 1-m^2 quadrat was circular and made of polycarbonate; the 10-m^2 quadrat was rectangular and made of ropes. We tested whether different quadrat sizes yielded different density estimates by sampling six depth-transects with both quadrat sizes and compared the estimates with a paired *t*-test.

Crayfish often exhibit variable distribution along a depth gradient (Abrahamsson and Goldman 1970; Momot and Gowing 1972; Capelli and Magnuson 1976; and Fig. 1). To ensure a representative sample of the populations, replicated quadrat samples were taken at different depths along transects perpendicular to the shore (Capelli and Magnuson 1976). The

[1]Contribution No. 324 of the Lake Memphremagog Research Project, McGill University Limnology Research Centre.

[2]Present address: Department of Zoology, University of Alberta, CW312 Biological Sciences Building, Edmonton, Alta. T6G 2E9, Canada.

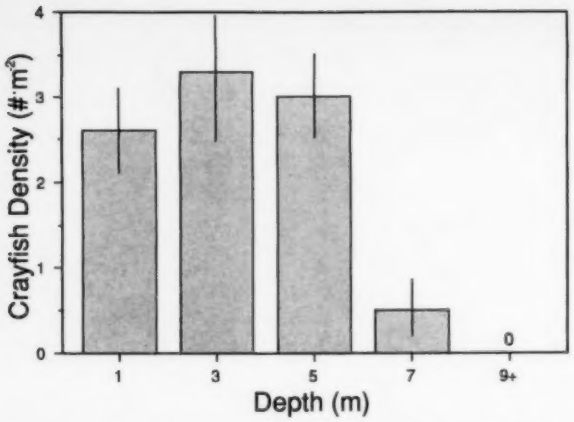

FIG. 1. Depth distribution of crayfish in MacPherson Bay, Lake Memphremagog, in June 1990. The error bars represent 1 SE. The thermocline was at approximately 8 m.

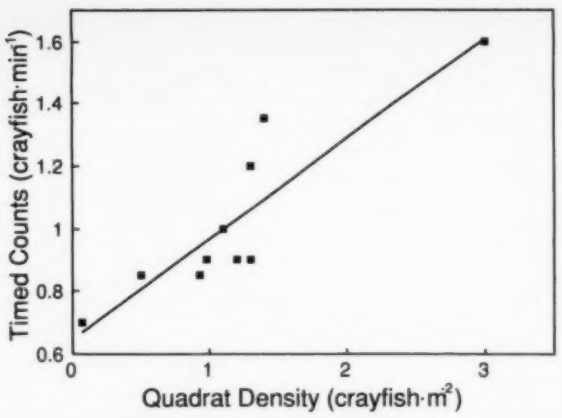

FIG. 2. Comparison of crayfish density estimates obtained by quadrat and timed counts for 10 transects from Lakes Magog and Memphremagog, southern Quebec. The relationship is described by the following linear regression: crayfish·min^{-1} = 0.65 + 0.32 crayfish·m^{-2}, $P < 0.01$, $R^2 = 0.79$, $n = 10$.

transects spanned from the shoreline down to the maximum depth where crayfish occurred, as determined by divers prior to quadrat sampling. Each rock within the quadrat was gently turned over and crayfish were collected by hand and transferred into a collection bucket (Davies and Ramsey 1989). All collections were made during daylight; throughout the study, only one crayfish was collected away from any obvious shelter. The number of crayfish caught and the number that were seen but escaped capture were recorded on a diving pad for each quadrat sample. The maximum depth where crayfish occurred and the distance from the shore were measured using marked ropes.

In addition to quadrat sampling, timed counts were measured for 10 transects from Lakes Memphrémagog and Magog, southern Quebec, prior to quadrat sampling. Two divers searched for as many crayfish as possible in 10 min. The number of crayfish observed was recorded but no attempt was made to catch them. As for quadrat sampling, young-of-the-year crayfish were not included in the counts. We compared timed counts with quadrat density estimates using linear regression.

Determining Sample Size

The first requirement to determine sample size is to obtain an estimate of the sampling variance (s^2) of the population (Zar 1984). In benthic invertebrate populations, the sampling variance is usually a function of the mean density ($\bar{X}$, number per square metre) and the quadrat size used (Q, square metres) (Downing 1977; Elliot 1979; Morin 1985). We used the mean and variance estimates collected in our survey to calculate a $\bar{X}$:s^2 relationship for crayfish. The relationship is best described by a log-linear function:

$$(1) \qquad \log_{10} s^2 = \log_{10} a + b \log_{10} \bar{X} + c \log_{10} Q.$$

The parameters a, b, and c are estimated with least-square regression (SAS Institute Inc. 1988). The back transformation of $\log_{10} s^2$ to s^2 can underestimate s^2 due to the error component left unexplained by the regression (Bird and Prairie 1985). A correction factor (CF) can be calculated using the residual mean square (RMS) of the regression as CF = $10^{(RMS/2)}$ (Bird and Prairie 1985).

The precision of a mean is defined by the size of its confidence interval, $\bar{X} \pm t_{\alpha(2),n-1}$ SE, with t the two-tailed Student t statistic for a given α and sample size $n - 1$ and SE the standard error of the mean (SE = $s/\sqrt{n}$). Therefore, at a given density the number of samples to collect will depend on (1) the desired width of the confidence interval, (2) the variability in the population (s^2), (3) the confidence level specified (α = 0.05, 0.01), and (4) statistical power ($1 - \beta$), the assurance that the confidence interval will be no larger than specified (Zar 1984). Statistical power is often overlooked in the planning of sampling programs (Downing and Downing 1992) and is especially important when the estimates are compared with parametric statistical tests (Zar 1984; Peterman 1990). With an approximate estimate of mean crayfish density, the following equation can be used to determine the number of quadrats to sample:

$$(2) \qquad n = \frac{s^2 \, \mathrm{CF} t^2_{\alpha(2),\,(n-1)} F_{\beta(1),\,(n-1,\nu)}}{(2 P \bar{X})^2}$$

(modified from Zar 1984) where s^2 is the variance estimated from the $\bar{X}$:s^2 function, CF is the correction factor for the back-transformation of log s^2, $t_{\alpha(2),\,(n-1)}$ is the two-tailed t value for a given α at $n - 1$ degrees of freedom, and $F_{\beta(1),\,(n-1,\nu)}$ is the one-tailed F value for a given β for $n - 1$ and ν degrees of freedom (with ν the sample size of the $\bar{X}$:s^2 regression). P, the ratio of SE/$\bar{X}$, defines the width of the confidence interval. For α = 0.05 and β = 0.1, P = 0.2 defines a 95% confidence interval no larger than ±40% of the mean 9 times out of 10. Because n is on both sides of the equation, the solution must be solved iteratively (Appendix). We have estimated the number of replicates to collect at different crayfish densities at α = 0.05 and β = 0.1, for P = 0.2, 0.3, and 0.5, for both quadrat sizes.

Sampling Effort

Sampling effort was defined as the amount of diver·hours required to collect the desired number of quadrat samples. In our survey, the number of quadrat samples that could be col-

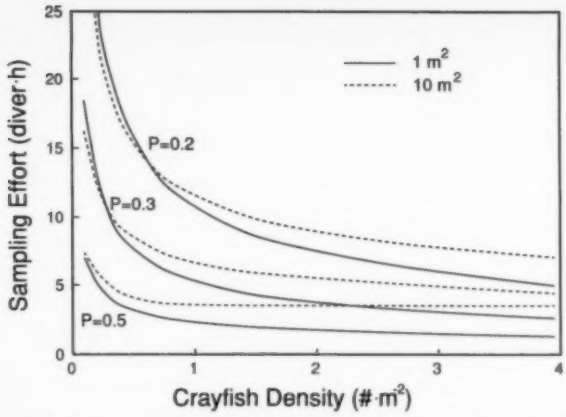

FIG. 3. Sampling effort required to obtain precise crayfish density estimates with a 1- and 10-m^2 quadrat. Three levels of precision are shown; these represent 95% confidence intervals no larger than ±40, ±60, or ±100% of the mean 9 times out of 10.

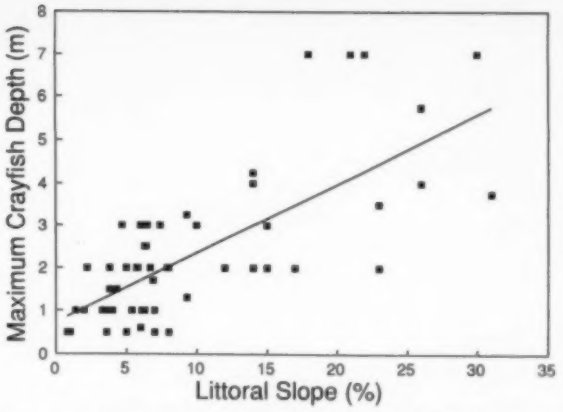

FIG. 4. Relationship between the maximum depth distribution of crayfish in lakes and the slope of the littoral zone. The relationship is described by the following regression: max. depth(m) = 0.73 + 0.16 slope (%), $P = 0.02$, $R^2 = 0.55$, $n = 57$.

TABLE 1. Summary statistics of the multiple regression model $\log_{10}s^2 = 0.215 + 1.28 \log_{10}\bar{X} - 0.742 \log_{10}Q$ where s^2 is the sampling variance, $\bar{X}$ is mean crayfish density (no.·m^{-2}), and Q is quadrat size (m^2). SE_i is the standard error of the regression coefficients, RMS is the residual mean square of the regression, CF is the correction factor to multiply to backtransformed values of $\log_{10}s^2$, R^2 is the coefficient of determination, and n is the number of transects sampled (36 with a 1-m^2 quadrat and 25 with a 10-m^2 quadrat). All t and F values are significant at $P < 0.0001$.

	SE_i	t or F value	RMS	CF	R^2	n
$\log_{10}\bar{X}$	0.0734	$t = 17.6$				
$\log_{10}Q$	0.0910	$t = -8.16$				
Regression		$F = 241$	0.113	1.14	0.89	61

TABLE 2. Number of quadrat samples needed to achieve precise crayfish density estimates at different levels of precision ($P = 0.2$, 0.3, and 0.5). All calculations made for $\alpha = 0.05$ and $\beta = 0.1$ (see Equation 2). The P values represent 95% confidence intervals no wider than ±40, ±60,or ±100% of the mean 9 times out of 10. The Appendix presents an example of the calculations.

Density (no.·m^{-2})	$P = 0.2$		$P = 0.3$		$P = 0.5$	
	1 m^2	10 m^2	1 m^2	10 m^2	1 m^2	10 m^2
0.10	>75	>25	>75	>25	56	14
0.25	>75	>25	>75	18	31	9
0.50	>75	23	50	13	21	6
1.0	65	16	32	9	14	5
2.0	42	11	21	7	10	5
4.0	27	8	14	5	7	4

lected per hour of SCUBA diving was roughly related to crayfish density:

(3) 1 m^2: Quadrat·diver^{-1}·h^{-1} = $6.43 - 1.62 \log_{10}\bar{X}$

$P = 0.005$, $R^2 = 0.23$, $n = 36$

(4) 10 m^2: Quadrat·diver^{-1}·h^{-1} = $1.43 - 0.48\log_{10}\bar{X}$

$P = 0.005$, $R^2 = 0.34$, $n = 25$.

We have compared the sampling effort required with each quadrat size at different crayfish densities at $\alpha = 0.05$ and $\beta = 0.1$ for three levels of precision ($P = 0.2$, 0.3, and 0.5).

Results and Discussion

There was no significant difference between density estimates obtained with different quadrat sizes for the six transects where both quadrats were used (paired t-test, $t_{0.05(2),5} = 0.114$, $P > 0.5$). Therefore, we did not introduce a systematic bias by sampling with different quadrat sizes. There was a linear relationship between timed counts and quadrat samples, but timed counts appeared less accurate (Fig. 2). For a 10-fold range in density obtained by quadrats, timed counts only showed a twofold range. Timed counts can be used to obtain an estimate of crayfish density prior to quadrat sampling, or provide a relative measure of abundance when precise estimates are not needed. Longer timed counts may be necessary when crayfish are collected (not only observed as we did) to compensate for the time spent catching them.

The sampling s^2 of crayfish populations is a positive function of $\bar{X}$ and negative function of Q:

(5) $\log_{10}s^2 = 0.215 + 1.28 \log_{10}\bar{X} - 0.742 \log_{10}Q$.

This relationship explains 89% of the variability in s^2 and the two predictors are highly significant (Table 1). The slope of $\log_{10}\bar{X}$ is lower than the average of 1.5 for other aquatic taxa, but crayfish follow the general rule that s^2 is greater than $\bar{X}$ once the effect of quadrat size is removed (Downing 1979; Morin 1985). Downing (1979) and Morin (1985) also found that larger quadrats reduce sampling variance for lake and river benthos.

Fewer quadrat samples are needed at all densities with a 10-m^2 quadrat because the estimates have lower variance (Table 2). However, a 1-m^2 quadrat is more efficient at densities above ≈0.3 crayfish·m^{-2} because more samples can be collected per unit effort (Fig. 3). This threshold, however, varies with precision; at high precision, it is advantageous to switch to a smaller quadrat at higher densities (Fig. 3). At low crayfish densities ($\bar{X} < 0.1$), both quadrat sizes require a lot of effort

to obtain precise density estimates. Belt-transects (i.e. crayfish collected within 1 m on each side of a transect line) may be more practical than quadrat sampling at low crayfish densities, especially if few rocks and boulders have to be turned (Quinn and Janssen 1989).

Depth Distribution

In most lakes, we only collected crayfish relatively close to the shore (from 0 to 50 m), with the maximum abundance often at the shoreline. The maximum depth where crayfish were collected was related to the slope of the bottom (Fig. 4), transects with steeper slopes having crayfish as far as the thermocline. Regardless of littoral slope, crayfish were seldom collected below the thermocline, presumably because of cold water temperatures (Abrahamsson and Goldman 1970; Momot and Gowing 1972; Capelli and Magnuson 1976). For transects with large variability in crayfish depth distribution, it is preferable to separate the littoral zone into two or more strata and sample each stratum separately to avoid inflating the sampling variance.

Our analysis concentrated on sampling crayfish at discrete sites or transects. The number of transects to be sampled within a lake to obtain a total population estimate would depend on the size of the lake and the variability in habitats along the lake perimeter. Because crayfish density varies widely within lakes (Davies 1989), stratified sampling based on distinct habitat types would be an efficient sampling strategy for a whole-lake population estimate (Cochran 1977; Davies 1989).

Acknowledgements

We thank B. Libman, E. Mellina and J. L. Sénécal for their help during field collections. J. Curtis, B. Parker, D. W. Schindler, I. Davies, and W. T. Momot provided helpful criticisms on early drafts of the manuscript. This work was supported by an NSERC operating grant to J. B. Rasmussen.

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Appendix

Sample calculation for the determination of sample size from Equation 2. We want to sample a site with a crayfish density of approximately 2 individual·m^{-2} using a 1-m^2 quadrat, and we want the 95% confidence interval ($\alpha = 0.05$) no larger than $\pm 60\%$ of the mean ($P = 0.3$) 9 times out of 10 ($\beta = 0.1$). To estimate s^2, we use Equation 5 and its correction factor CF for the backtransformation from $\log_{10} s^2$ (Table 1). Because n is on both sides of the equation, we must guess an initial value, say $n = 25$:

$$n = \frac{10^{(0.215 + 1.28\log_{10}\bar{X} - 0.742\log_{10}Q)}\mathrm{CF}t^2_{0.05(2),24}F_{0.1(1),(24,61)}}{(2PX)^2}$$

$$= \frac{10^{0.600}\cdot 1.14\cdot 2.064^2\cdot 1.51}{(2\cdot 0.3\cdot 2)^2} = 20\,.$$

We then reiterate the equation using $n = 20$ to determine the degrees of freedom of the new t and F statistics. The solution converges at $n = 21$.

Impact Assessment Using the Before-After-Control-Impact (BACI) Model: Concerns and Comments

Eric P. Smith

Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

and

University Center for Environmental and Hazardous Materials Studies, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

and David R. Orvos[1] and John Cairns, Jr.

University Center for Environmental and Hazardous Materials Studies, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Smith, E. P., D. R. Orvos, and J. Cairns, Jr. 1993. Impact assessment using the before-after-control-impact (BACI) model: concerns and comments. Can. J. Fish. Aquat. Sci. 50: 627–637.

The effect of a change in an ecosystem can often be assessed through the use of a statistical model that incorporates the change. A sensible approach for assessing the effects of an industrial or power plant on the aquatic environment is to sample the environment both before and after the plant starts operation and test for a change in some biologically relevant parameter. To improve sensitivity, samples may be taken at a control site as well as at sites receiving the plant effluent. While this provides a powerful means for assessing effects, the implementation of the design is important and subsequent analysis of the collected data depends on proper implementation. Problems such as trends in the measurements, failure to meet the assumptions of the model, irregular sampling, confounding factors, and changes in the habitat can influence results, as we illustrate using a long-term impact assessment of a power plant on fish populations. In long-term studies, it may be difficult to separate effects due to the plant from effects due to other sources. Sound design requires both a good statistical model and an understanding of the underlying biological processes (what to measure) and careful planning (how to measure it well).

Les effets d'un changement survenant dans un écosystème peuvent souvent être évalués par l'utilisation d'un modèle statistique qui englobe ce changement. Une façon logique d'évaluer les effets d'une usine ou d'une installation de production d'énergie consiste à prélever des échantillons dans le milieu avant et après le début des opérations et à réaliser un test portant sur la variation d'un paramètre biologique pertinent. La sensibilité peut être accrue en prélevant des échantillons dans un site témoin et dans des sites récepteurs des effluents de l'usine. Cette façon de procéder est un très bon moyen d'évaluer les effets, mais la mise en oeuvre d'un tel protocole doit être faite de façon adéquate car elle influe sur les résultats de l'analyse des données recueillies. Nous illustrons les effets de certains problèmes, tels les tendances des mesures, l'impossibilité de se conformer aux hypothèses du modèle, l'irrégularité de l'échantillonnage, les facteurs à effets confondus et la modification de l'habitat, sur les résultats de par une évaluation des incidences à long terme d'une usine de production d'énergie sur les populations de poisson. Il peut s'avérer difficile, au cours des études à long terme, de distinguer entre les effets de l'usine et ceux d'autres sources. Une bonne conception suppose l'utilisation d'un modèle statistique approprié, la connaissance des processus biologiques sous-jacents (à mesurer) et une planification méticuleuse (comment mesurer adéquatement).

Received December 11, 1991
Accepted September 14, 1992
(JB330)

Reçu le 11 décembre 1991
Accepté le 14 septembre 1992

Measuring and assessing the impact of industrial operations on the environment are of great importance because recent changes in the legal and socioeconomic environment make determination of impact crucial to regulatory compliance and assessment of potential risk. Whenever a new industrial plant begins operation, the effect of the operation upon the ecosystem must be assessed. However, the design and analysis of ecological monitoring studies are not simple tasks unless the effects of the suspected impact are great and well defined. A number of articles have discussed the design of ecological impact assessments (Green 1979, 1989; Hurlbert 1984; Peterman 1990; Eberhardt and Thomas 1991).

The simplest design for impact assessment was presented in Green (1979). In this approach, potential ecological impacts are assessed by collecting data in a control and impact zone both before and after a potential impact begins. The impact could then be assessed using a two-factor analysis of variance (or multivariate extension for multivariate observations). Hurlbert (1984) has criticized ecological impact assessment methods (using as an example the above design) as not being sound from a statistical design view, but did not suggest alternatives. The problem that Hurlbert identified was one of lack of replication and the inability to randomize the impact to the sites. Stewart-Oaten et al. (1986), in a rejoinder to Hurlbert, suggested a

[1]Present address: Environmental Research Laboratory, SmithKline Beecham Corporation, 709 Swedeland Road, King of Prussia, PA 19406, USA.

TABLE 1. Data for the standard BACI analysis.

Treatment	Time	Control	Impact	Difference
Before	1	y_{b1c}	y_{b1I}	d_{b1}
	2	y_{b2c}	y_{b2I}	d_{12}
	.	.	.	.
	.	.	.	.
	.	.	.	.
	n	y_{bnc}	y_{bnI}	d_{bn}
After	$n + 1$	y_{a1c}	y_{b1I}	d_{a1}
	$n + 2$	y_{a2c}	y_{b2I}	d_{a2}
	.	.	.	.
	.	.	.	.
	.	.	.	.
	$n + m$	y_{amc}	y_{bmI}	d_{am}

design involving data on pairs of stations, one viewed as control and the other in the impact zone, collected both before and after the onset of the potential impact. This design differs from the simple two-factor design in that the control and impact sites are paired. The design is quite similar to the one used by Thomas et al. (1978), and both researchers used the design to assess effects of nuclear power plants.

This paper discusses the before-after-control-impact (BACI) design and describes some concerns that were realized during attempts to apply the design to assess the impact of a nuclear power plant on a river community. Some of the problems encountered that make the general task of assessing potential impact effects on organisms difficult are described. Recently, Stewart-Oaten et al. (1992) have discussed some potential problems with impact assessment. While their approach dealt primarily with the theoretical aspects of design and the resulting model, our approach focuses on a case study and specific problems encountered in assessments using fish in a river system. Some of the problems are specific to the BACI design; however, many are common to the more general problem of ecological impact assessment and even long-term ecological studies.

The BACI Approach

The BACI design (as described in Stewart-Oaten et al. 1986) requires data on two sites, corresponding to a control site and an impact site. Data are collected a number of times before the impact begins as well as after. Thus, there are two treatments: before-after, which is of primary interest, and control-impact, which is of secondary interest. Data for this analysis are summarized in Table 1. The sites are viewed as pairs and as forming a block in time. Data as described in Table 1 suggest analysis using a split-plot model (Milliken and Johnson 1984). The whole plots are the times of sampling and the split plots are the locations within the times. The model for the data is given by

$$y_{ijk} = \mu + \alpha_i + t_{ij} + \beta_k + (\alpha\beta)_{ik} + \epsilon_{ijk}$$

where μ is the overall mean, α_i is the effect associated with before-after ($i = b,a$), t_{ij} is the factor associated with time of sampling (block) ($j = 1,2, \ldots, n$ for $i = b$ and $j = 1,2, \ldots, m$ for $i = a$), β_k is the effect associated with control-impact ($k = c,I$), $(\alpha\beta)_{ik}$ is the interaction term, and ϵ_{ijk} is the error term. The analysis of variance table for this design is given in Table 2. Three tests are associated with this analysis, corresponding to two main effects (before-after and control-impact) and the interaction. The BACI test (when applied as a t-test, not a Mann–Whitney test) is equivalent to the test for an interaction effect (specifically, the BA × CI interaction). The above parametric approach is similar to a model used by Thomas et al. (1978) in the analysis of impacts at nuclear power plants.

TABLE 2. ANOVA table for BACI analysis.

Source	Sum of squares	Degrees of freedom	Mean square	F statistic
Before-after	SSBA	1	MSBA	MSBA/MSE1
Error 1	SSE1	$n + m - 2$	MSE1	
Control-impact	SSCI	1	MSCI	MSCI/MSE2
BA × CI	SSBACI	1	MSBACI	MSBACI/MSE2
Error 2	SSE2	$n + m - 2$	MSE2	
Total	SST	$2n + 2m - 1$		

Stewart-Oaten et al. (1986) suggested an alternative analysis that is analogous to the use of a paired t-test instead of a randomized block analysis when there are two treatment levels (e.g. see Kleinbaum et al. 1988, p. 392). Differences are formed between the control site and the impact site. These differences are then analyzed for a before-after treatment effect. Because the differences now appear as two samples of data (Table 1), the approach to the analysis is similar to that used for standard two-sample tests. The recommended approach is to use a two-sample t-test or a nonparametric test, such as the Wilcoxon rank sum or Mann–Whitney test, to assess change. If the two-sided t-test is used, the p-value is the same as that of the F-test for interaction (BA × CI effect in Table 2). The nonparametric approach is recommended to reduce the effects of extreme data values and failures in the assumptions that occur in most ecological data.

One potential problem with blocking designs is interaction between the blocking factor and the treatment factors, which is commonly referred to as nonadditivity. The recommended procedure also suggests transforming the data prior to differencing to achieve additivity. In the BACI analysis, additivity means that the differences in the control site are roughly parallel to those in the impact site, i.e. the shape of the plot of one site against time is similar to the plot of the other site except for a shift. The test is computed by testing the correlation between two series generated from the before period: one calculated as the sum of the measured values at the two sites at each sample time and the other calculated as the difference of these values.

Additivity is usually violated in one of two ways. First, the sites may differ in scale. A plot of the control site abundance versus time may resemble the impact site, but with more (or less) variability. Thus, one site may have both a larger mean and a larger variance. The second violation of additivity occurs when there is a trend in the observations in the before period for one of the sites or different trends in the observations at the different sites. These types of trends would suggest that the differences in the before period are not roughly constant but change with time. The first violation of additivity (scale) can often be removed by using a transformation. The second violation (trend) may not be removed using a transformation (especially the one that removes nonadditivity) and may lead to misleading significance (see fig. 3 in Stewart-Oaten et al. 1986). Stewart-Oaten et al. (1986) stated "Thus no attempt should be made to choose a transformation to correct this change. Rather, such correction should be avoided, the trend should be tested for, and the species not used in this analysis if the trend is present." If the same trend is present in control and impact sites, this would not violate the assumption of additivity, as this trend situation would imply parallel lines and con-

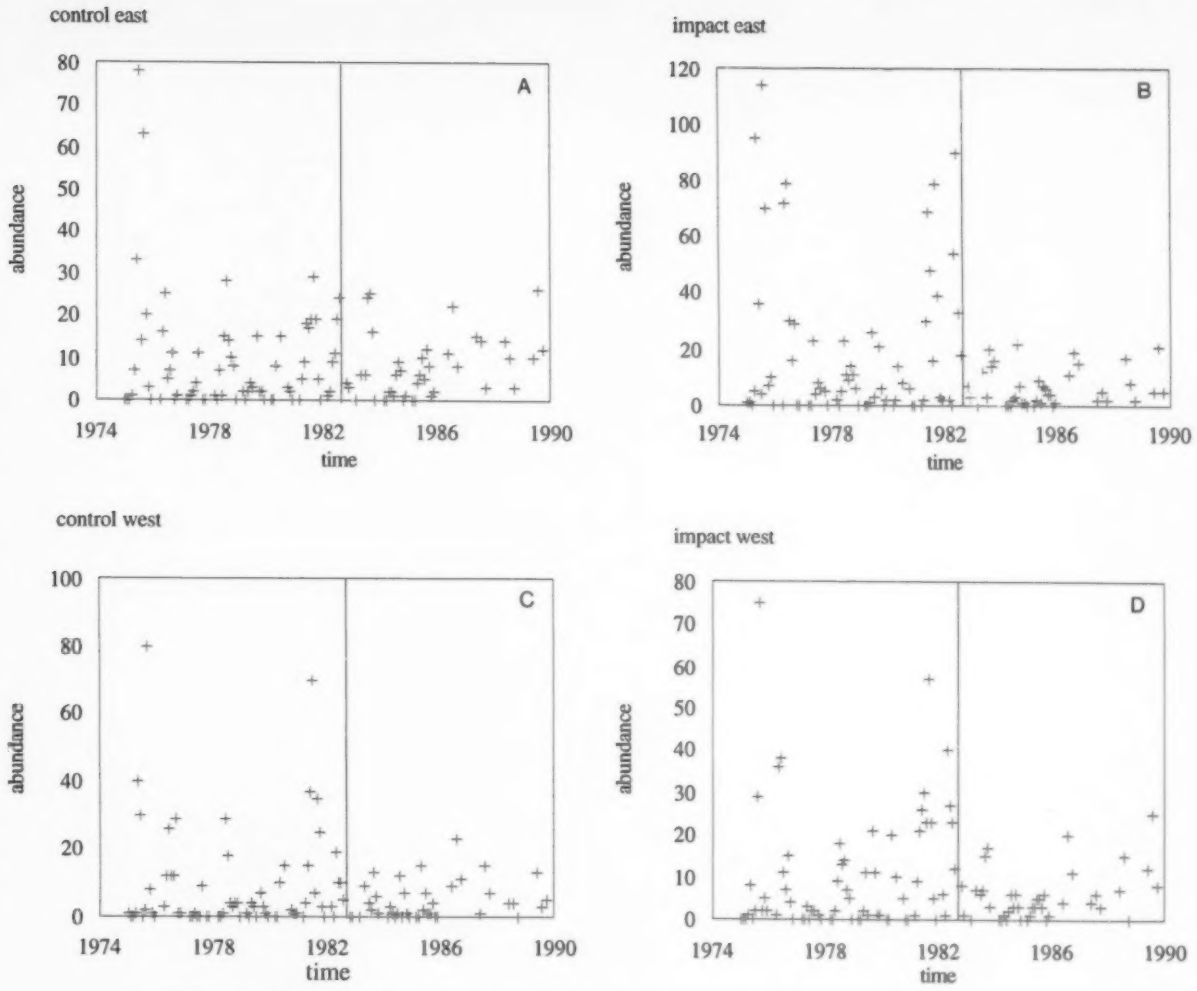

FIG. 1. Plot of the abundance of taxon 14 for (A) control east, (B) impact east, (C) control west, and (D) impact west.

stant differences between the lines. However, the presence of trend would have to be incorporated into the model to make accurate inferences.

To examine the utility of the BACI model, an actual case study is used in which data were collected both below and above a plant that discharged cooling water into a second-order river. Data were collected both before and after the plant began operation. The data set included information on a number of chemicals, phytoplankton, and benthic and fish taxa. In the fish study, data were collected using electrofishing at both impact and control sides of the river on 19 specific taxa; all remaining fish were classified into an unidentified group. Data were collected at two locations (east and west sides of the river) for almost 7 yr prior to plant operation and for 7 yr afterward (the start of the plant was in September 1982). Data from the two sampling sites at each location (east and west side of the river) initially were pooled to create one sample site at each location as suggested by Stewart-Oaten and his colleagues. Data for one of the 20 fish taxa are shown in Fig. 1 for each side of the river to illustrate some of the concerns. The data analyzed are the total catch from the electrofishing. Roughly equal effort (time and space) was expended for each collection, so the data were not adjusted for effort. The focus of this discussion is the analysis of the fish at this site and whether the plant is responsible for any change in fish abundance.

Table 3 presents the results of applying the BACI model to the fish species data. The additivity test based on Pearson's correlation coefficient was carried out on five transformations (none, square root, log, inverse square root, and inverse). A constant was added to the abundance prior to transformation (0.5 for the square root and 1.0 for the log, inverse square root, and inverse transformations). The transformation that gave the most nonsignificant p-value for additivity was used. After transformation, the Wilcoxon test was used on the differences to assess effect. Table 3 (the first column of tests) indicates that there are significant effects for five of the fish taxa. Thus, the preliminary conclusion is that the plant has a significant impact on the fish community ($\alpha = 0.05$). It may be the opinion of some readers that the testing should be multivariate or adjusted in some manner for the number of tests that are run, as a large

TABLE 3. Results of applying the BACI model to the 20 fish taxa. ***Significant at the 0.05% level (Wilcoxon test). Transformation used to correct for nonadditivity is given in parentheses following the *p*-value (S = square root, L = log, IS = inverse square root, I = inverse). Samples sizes are 70 for the before period and 41 for the after period. + + + indicates that none of the transformations was nonsignificant in the additivity test.

Taxon number	*p*-value using all the data	Sample sizes, excluding joint zeros	p-value excluding joint zeros
1	0.0037 (I)***	49, 21	0.0095 (I)***
2	0.7231 (IS)	62, 36	0.5383 (IS)
3	0.1493 (IS)	48, 26	0.1520 (IS)
4	0.0574 (IS)	70, 41	0.0574 (IS)
5	0.0051 (IS)***	63, 34	0.0014 (L)***
6	0.0367 (L)***	67, 40	0.0442 (S)***
7	0.5311 (I)	40, 9	0.0047 (I)***
8	0.4043 (I)	37, 28	0.2255 (I)
9	0.1244 (IS)	58, 37	0.0464 (L)***
10	0.0003 (IS)***	61, 37	0.0003 (S)***
11	0.9976 (S)	12, 11	0.9755 (L)
12	0.5971 (I)	29, 16	0.5693 (I)
13	0.2149 (I)	41, 28	0.3856 (I)
14	0.0201 (IS)***	62, 38	0.0197 (L)***
15	0.7716 (I)	26, 13	0.4839 (I)
16	+ + +	24, 28	0.0356 (I)***
17	+ + +	32, 16	0.8783 (I)
18	0.9659 (I)	32, 20	0.7349 (I)
19	0.7048 (IS)	69, 41	0.6969 (IS)
20 (unidentified)	0.1360 (L)	57, 41	0.0109 (IS)***

number of taxa are analyzed and the species abundances may not be independent. If a simple Bonferroni correction is used, significance would result when the *p*-value is less than 0.0025. Only one species (10) would be significant using this approach. One drawback to this approach is that the importance of the species is not accounted for. Species 14, for example, is a popular game fish. Further, if the number of taxa is large, the sensitivity of the inference may be compromised. The Bonferroni method is a conservative approach and, as such, results in industry being favored over the environment. Our preference is to consider the taxa on a more individual basis.

Concerns and Comments

While the BACI analysis represents an improvement over many other analyses that are or have been used, questions arise about its performance and the ability to assign cause to change in long-term biological impact assessments. During the course of analyzing the data from our case history, several concerns became apparent.

Zeros in the Data Set

The first difficulty encountered with the data and the model arose because of zeros in the data set. Zeros correspond to either an absence of the fish in the area at the time of sampling or an absence due to low sampling intensity. For example, the ability to catch fish using electrofishing at this particular site is different in winter than in summer. Furthermore, the winter months tend to have lower abundances, as the young fish of many species appear in spring. This may result in more zero data in winter than in summer. The difficulties in analyzing data with zeros are similar to the difficulties encountered when analyzing censored data (e.g. in chemical measurements). The estimates of parameters are affected, tests tend to have reduced power, and models do not adequately describe the data (see Helsel 1990 for a review of censoring and Lambert 1992 for a method for analyzing data with zeros). Figure 2 shows the differences (control-impact) for species 14; many of the differences are zero. The differences that are zero come from cases that were jointly zero and also cases where the abundances were small and of the same value (see also Fig. 1). Differences were quite large and negative during two intervals in the before data. This reflects sampling times where the abundance was large in the impact area but small in the control area. These large differences do not occur in the after period, however, and suggest an impact effect. Why the differences are so large is also of interest.

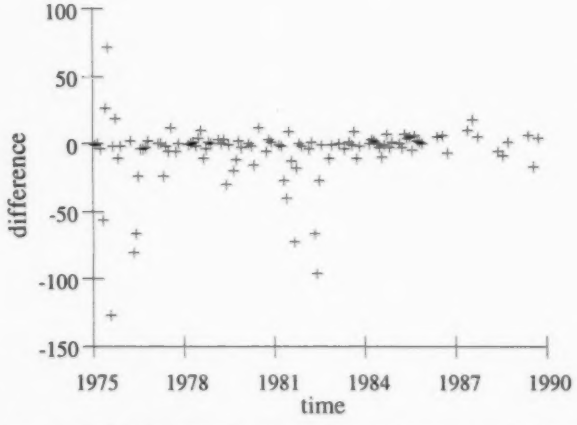

FIG. 2. Plot of the difference (control-impact) between control sites and impact sites for taxon 14.

One simple approach to dealing with zeros is to drop the sampling times in which zeros occur in both the control and impact sites. The results of applying the BACI model to fish data without joint zeros are given in the last column in Table 3. There are several more species that show significant changes, including the unidentified category. The increase in the number of significant species and a review of respective scatterplots indicate that the effect of the zeros is to increase the variance and make the means closer to zero. Some species have a large number of joint zeros (species 7, 11, and 15–18 in Table 3). Models to account for zeros are available for simple situations but present computational difficulties for models such as the split-plot model (Lambert 1992).

Additivity

A second concern with transformations is the relationship between the transformation to achieve additivity and the use of a nonparametric procedure to test for a plant effect. As biological field data tend to have nonnormal qualities, nonparametric methods are useful. Two concerns arise in their use. First, a philosophical problem is that the nonparametric test is applied because the researcher believes that a parametric procedure will not be valid; however, the test for additivity and choice of transformation depend on a parametric test. Second, the test for additivity, being based on a parametric analysis, will be sensitive to violations of some of the assumptions. What is the effect of using the nonparametric test after a transformation is chosen based on a parametric procedure when there are outliers or trends in the data? What are some other approaches to adjust for this problem and are they effective? Transformations such as the inverse and inverse square root may deemphasize the large values and overemphasize the small values (especially the zero values). What are the effects of zero values with these and other transformations on the nonparametric test? It is crucial that these questions receive further research.

A further philosophical concern with the BACI analysis is the test for additivity. As indicated in Table 3, all the fish species data required transformation, except for two for which additivity could not be achieved using any of the four transformations. Many transformations are either the inverse or inverse square root transformations. The test used in Table 3 is for only one type of violation of additivity: scale differences between the control and impact areas. In fact, the test is equivalent to testing equality of variance for the data from the control and impact areas in the before period (see Bradley and Blackwood 1989). Other forms of nonadditivity, in particular, that of trends in the differences in time, should be investigated in a BACI analysis (as noted by Stewart-Oaten et al. 1986). Trends may occur in the differences or in the individual locations.

Sampson and Guttorp (1991) noted that, if a transformation is made, then this transformation also affects the hypothesis being tested. The hypothesis of no impact effect in the original scale is not the same as the hypothesis of no impact effect in a transformed scale. For example, a test of no impact in the original scale corresponds to a test on the mean and variance in the inverse scale (x^{-1}), not a test on the means in the inverse scale. Furthermore, the BACI test of no interaction in the inverse scale may not mean that there is no interaction (impact) in the original scale. Thus, interpretation of the data may be confounded by the transformation. Sampson and Guttorp (1991) presented an approach for testing hypotheses about impacts in the original scale using data in a transformed scale.

Some of these difficulties are illustrated in Table 4. Several transformations are applied and additivity and a test for impact conducted using both parametric and nonparametric methods. This produced two interesting results. First, for both the combined data set and the river east side data set, the largest p-values for additivity tests are the inverse square root transformation and the inverse transformation, respectively, regardless of whether a parametric or nonparametric method is used. However, the parametric and nonparametric tests for impact give different results. In both cases, the nonparametric method results in significance, while the parametric test is not significant. The second concern occurs with the test of additivity for the river west side. The largest p-value using a parametric test is for the square root transformation. However, this transformation is significant (at the 0.05 level) using a nonparametric method. The nonparametric approach suggests using a log transformation.

Data transformation is also useful for normalizing the data and making the assumptions for parametric analysis more tenable. However, transformations do not work well with all distributions. Data used here are count data, specifically the number of fish of a particular species. When a large number of observations have the same value (in this case, zero), the transformation may not work as anticipated and will not remove the effect of the cluster of observations with the same value. Stewart-Oaten et al. (1992) further noted that transforming to achieve additivity may not always succeed and that such a failure may indicate that the treatment effect changes with another factor, for example, season.

Assumption of Independence

Another difficulty with the BACI analysis is the assumption of independence. While some evidence shows that the Mann–Whitney test is less sensitive to the independence assumption (Hirsch et al. 1982), it is still affected. Furthermore, the test of additivity may be sensitive to temporal dependence (serial autocorrelation). Temporal dependence could cause an excessive number of rejections. Seasonality may also be a problem because fish populations undergo natural fluctuations over a year. Differences in fish abundance in December may have quite different statistical properties than differences in June when the number of young fish increases. The BACI model is most effective if the variance in the difference does not change over sampling time. This is probably not true for fish in temperate rivers because the variance in the summer difference may be substantially higher than the variance in the winter months. Methods for analyzing impact data with seasonal effects are available (Thomas et al. 1978; Hirsch et al. 1982; van Belle and Hughes 1984).

Sampling Frequency

The BACI model works best if the timing of sampling is the same in the before and after periods. For example, if, during the before period the frequency of sampling is high, with samples taken on a monthly basis, but in the after period, sampling occurs on a monthly basis for some years and only in the summer months thereafter, there may be fewer small differences in the after period than in the before period (as winter months are excluded). For the power plant data, samples were taken approximately once each month for most of the study. However, in the last 3 yr, sites were sampled only in June, August, and October (Fig. 1). This unbalancing of data may cause variances

TABLE 4. *p*-values for additivity and the BACI test for a variety of transformations of data on taxon 14. Additivity is tested using a parametric (Pearson) and nonparametric (Kendall) correlation. The test for impact is carried out using a two-sample *t*-test (equal variances) and the Wilcoxon two-sample test. The analyses are done for each sampling site and for the combined sites. *Optimal transformation.

Transformation	Combined	East	West	Test
				Additivity
None	0.0001	0.0002	0.7927	Pearson
	0.0001	0.0002	0.0135	Kendall
	0.0024	0.0008	0.5366	*t*-test
	0.0030	0.0008	0.3843	Wilcoxon
				Additivity
Square root	0.0001	0.0001	0.8613*	Pearson
	0.0003	0.0001	0.0454	Kendall
	0.0011	0.0002	0.6867	*t*-test
	0.0031	0.0015	0.4723	Wilcoxon
				Additivity
Log	0.5243	0.0232	0.8108	Pearson
	0.3524	0.0139	0.7875*	Kendall
	0.0677	0.0024	0.9314	*t*-test
	0.0098	0.0035	0.9490	Wilcoxon
				Additivity
Inverse square root	0.9454*	0.3918	0.7728	Pearson
	0.8837*	0.4683	0.1702	Kendall
	0.1687	0.1021	0.3743	*t*-test
	0.0265	0.0089	0.6447	Wilcoxon
				Additivity
Inverse	0.7172	0.5812*	0.5862	Pearson
	0.3714	0.9935*	0.0751	Kendall
	0.3742	0.4196	0.3280	*t*-test
	0.0434	0.0149	0.5497	Wilcoxon

in the after period to be larger than in the before period, thus affecting the tests and possibly resulting in detection of differences that are not there.

It is difficult to sample rivers on a common day each month because events such as floods and foul weather can severely affect the performance of sampling. Electrofishing is commonly done at the same depth; however, if the river is flooded, then the only locations with an appropriate depth may be on a site that was previously not underwater. The effects of minor inconsistencies in sampling frequency should have minor effects on the analysis. However, it is best to have the after sampling as similar as possible to the before sampling.

Pooling of Sample Sites

The question of how to analyze data collected at multiple stations (locations within the sites) in impact and control sites is difficult to answer. The simplest approach is to pool data from the locations. However, there are a number of concerns with this approach. Pooling data from the locations is valid if the locations within the sites do not significantly alter the abundance of fish. However, two sites in a river may be quite different due to differences in the soils, depth of the river at the site, or the habitats. Site variability can be very important if the control sites are different from the impact sites or if the impact affects sites differentially. Site differences can also increase statistical variability and make the test less sensitive. In our case history, the plant is located near a bend in the river, and the habitat below the plant is different from that above the plant, especially for the site located on the outside of the bend (in our example, the west site). The bend may cause the habitat at this outside site below the plant to be different from that on the similar site above the plant as well as the site directly across the river. Using the fish data, the analysis can be applied to each site pair. Alternatively, a site variable can be added to the model, although this would make the analysis more complicated. It would be appropriate to use the bank to pair the sites; however, if the outer site below the plant is quite different in terms of habitat, then it may be that neither of the control sites provides an adequate control for this particular site. This problem cannot be resolved by analytical methods.

Temporal Blocking

The BACI is a sensitive method for analyzing data. Because differences are used in the analysis, sensitivity to events occurring in both areas (e.g. seasonality, flooding effects) is diminished. However, in planning BACI studies, the design is important (see Eberhardt and Thomas 1991). The design treats the two sampling sites (above and below the plant) as a temporal block. The design is most powerful if the temporal blocks are true blocks and reduce variation. Variation is reduced by blocking if the sites within a block are similar (and remain similar over time). If the control site is notably different from the impact site, then the sites are likely to respond differently to natural changes in environmental conditions and, as a result, confound conclusions. For example, habitat may change due to natural fluctuations in river flow over long periods. If these river flow patterns are different in the control site as compared with the impact site, then the habitat change in the control site is likely to be different from that in the impact site. Hence, fish abundance would be expected to change differentially. If the flows tend to be higher in the before period than in the after period, then before-after is confounded with flow. This confounding

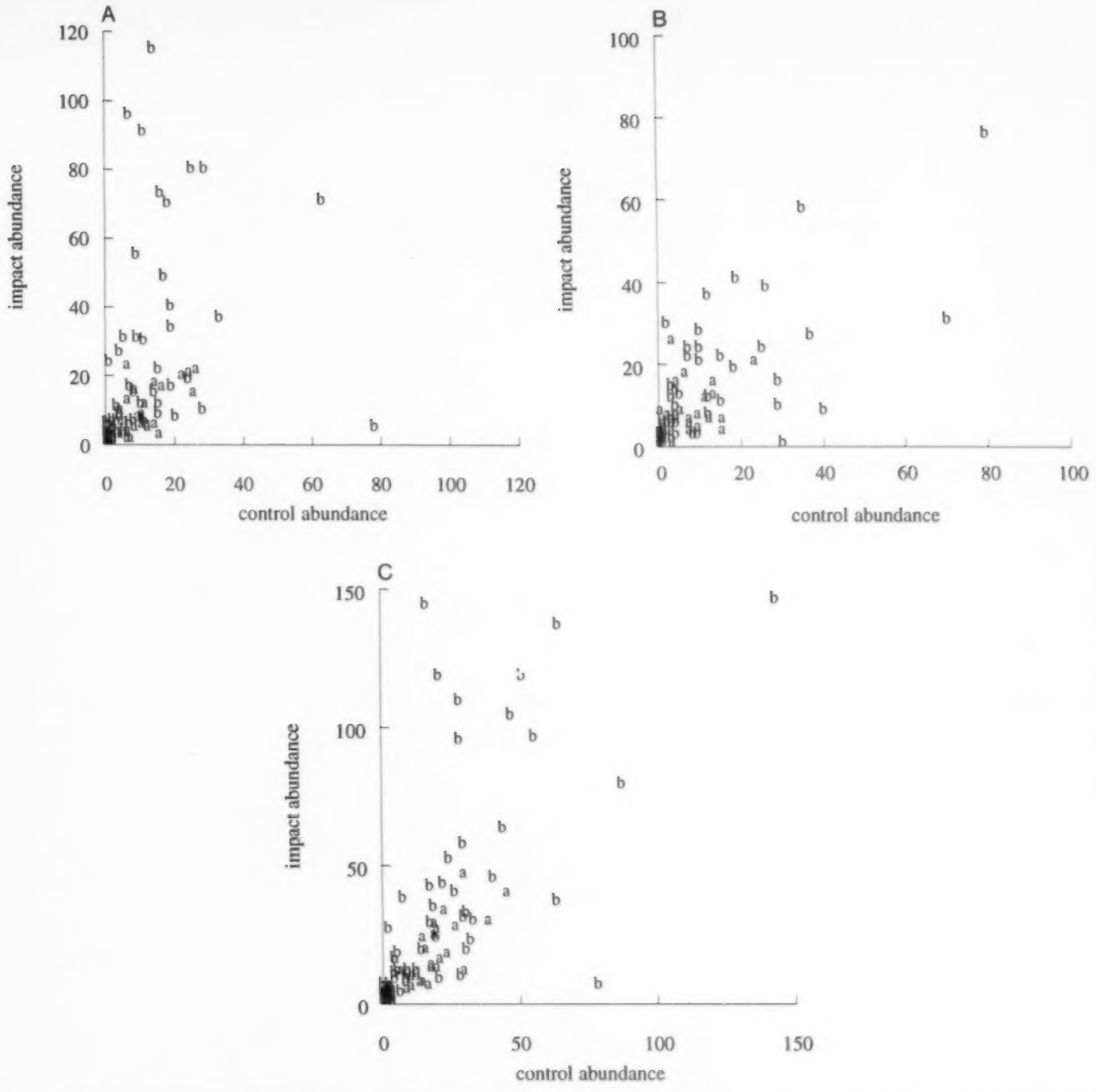

FIG. 3. Plot of the control site abundance versus the impact site abundance for taxon 14 for (A) east sites, (B) west sites, and (C) combined sites. The period is denoted b for before and a for after.

could be removed by adjusting the data for flow and then applying a BACI analysis. An indication of a flow effect could appear as a trend or some other pattern in the before data. While some of the effects of these factors can be removed, it requires planning (e.g. collect flow data from the start) and knowledge of the factor. Without planning and knowledge, the design may become flawed.

While it cannot be expected that dissimilar sites will change in an ecologically similar fashion, there is no guarantee that ecologically similar sites will remain ecologically similar over time (McCune and Allen 1985). Microhabitat differences in the sites may affect food chain dynamics and species abundances. Differential competition and predation may result in quite different species distributions.

A simple assessment of the blocking is obtained by graphing before data for the control site versus the impact site. In Fig. 3, the data for species 14 are plotted for both before (using the label b) and after (a). If the blocking is effective (i.e. greatly reduces variation), the data from the before period would be expected to fall near a straight line with a slope of 1 (but the intercept need not be zero). All three plots suggest that the blocking is not effective. Large deviations in the slope from 1

TABLE 5. p-values for test for trend in time for data from the before plant operation period using Kendall's tau statistic. The sign of the correlation is given in parentheses for the significant ($p < 0.05$) correlations. Cases with joint zeros in the control and impact were deleted.

Species	n	Control west	Control east	Impact west	Impact east
1	49	0.4903	0.8043	0.0004 (+)	0.7411
2	62	0.0637	0.0269 (+)	0.3110	0.2280
3	48	0.0542	0.6314	0.0646	0.5201
4	61	0.2377	0.5692	0.0097 (−)	0.0487 (−)
5	70	0.1752	0.0012 (−)	0.0464 (−)	0.0036 (−)
6	63	0.3875	0.9516	0.0007	0.8089
7	67	0.4920	0.4536	0.2618	0.0894
8	40	0.0031 (−)	0.4570	0.2143	0.0039 (−)
9	37	0.2215	0.1963	0.9214	0.6400
10	58	0.0717	0.0085 (+)	0.0000 (+)	0.0570
11	12	0.1111	0.3497	0.3364	0.9353
12	29	0.0823	0.6769	0.1979	0.4163
13	41	0.0006 (−)	0.2256	0.9130	0.0027 (−)
14	62	0.4733	0.4275	0.0478 (+)	0.9708
15	26	0.0901	0.0017 (−)	0.9090	0.0214 (−)
16	24	0.2931	0.5902	0.7089	0.1235
17	31	0.2586	0.1714	0.4811	0.0449 (−)
18	32	0.0016 (−)	0.0079 (−)	0.4586	0.6162
19	69	0.0003 (+)	0.0246 (+)	0.0000 (+)	0.0012 (+)
20	57	0.0004 (+)	0.0678	0.0174 (+)	0.0018 (+)

and low correlations suggest inadequate blocking. Figure 3A, in particular, indicates possible problems in that the high densities for impact are not matched with high densities in the control. Although the after data are plotted on the graphs, these values are difficult to observe because almost all of the values are small. Thus, there is a reduction of abundances not only for the impact area but also for the control area. The small differences noted in Fig. 2 for the after period are due to a reduction in both areas. Because of the lack of matching, it is difficult to ascertain if the effect is due to the operation of the plant or some other factor.

Confounding Factors

It is quite a leap of faith to assume that there are no other factors that influence the ecology of the system. The argument in favor of the BACI design is that, if the factor influences both the control and impact sites equally, the effect is removed by differencing. However, our experience with long-term studies suggests that factors affect sites differentially over time. The effects of these factors needs to be investigated. In the case of our example, the building of the plant and economic changes that result from plant construction may be an important factor. It may be quite difficult to disentangle the effect of construction from the plant operation, as these factors are confounded in time. In our example, a large number of confounding variables further suggests that the plant may not cause substantial ecological change. These include effects of a sewage treatment plant, addition of antifouling chemicals, differences in flow for different years, changes in the sampling plan, and changes in habitat. A bacterial insecticide (*Bacillus thuringiensis*) was applied in shallow areas starting in 1986 to control blackflies. The insecticide may affect macroinvertebrates and, hence, the food chain. Other indirect effects are also possible. The construction and operation of the plant require a large number of personnel that, in itself, could also have affected results. Increased fishing intensity in the impact area may have resulted in some significant changes. Data on these factors are needed to assess their influence. Then, the BACI analysis could be rerun, adjusting for the variables. Without this information, the results of the statistical analysis may be indefensible in a court of law. Even with the information, the analysis may be weakened.

Further Statistical Analysis of BACI Data

Because of the problems suggested in the above discussion, several additional analyses were conducted.

(1) A test of trend in the control and impact areas was conducted. As mentioned above, differences in trend in the control and impact area before plant startup in 1982 would be a violation in the assumption of additivity.

(2) An analysis of a reduced data set was conducted. Data were reduced by eliminating the first year of sampling (1975), which was a year of preliminary results. Also, because the final sampling was done only in June, August, and October, data from other months were deleted. This provided identical sampling times for both the before and after data (a more balanced data set).

(3) Separate analyses for the east and west sides of the river were conducted. The bend in the river would suggest that the sides are different and, hence, that pooling would not be appropriate.

(4) To examine variability in the before data, the BACI model was used on only the before data. Because it is important that the control be similar to the impact site prior to plant operation, we compared the control and impact sites using the BACI model with data from the period 1975–79 as the preimpact data and 1979–82 as the postimpact data. The month where the division of data was assigned was chosen to be similar to that of the full data set with the actual impact. One other split of the data was used to ascertain the influence of the split.

(5) Finally, an analysis of the presence and absence pattern of fish species in the samples was conducted. Species that are absent in a large number of samples are not useful indicators

TABLE 6. *p*-values for tests of trend in the difference in control and impact using Kendall's tau and the data for the before plant operation period. Cases with zeros in both the control and impact sites were deleted. ***Significant at the 5% level.

Species	Combined	East	West
1	0.0101***	0.8576	0.0008***
2	0.8837	0.7467	0.4544
3	0.7819	0.7356	1.00
4	0.0502	0.2161	0.0923
5	0.0072***	0.0268***	0.0150***
6	0.0063***	0.8751	0.0128***
7	0.1772	0.5728	0.4119
8	0.2084	0.0155***	0.1910
9	0.3466	0.6138	0.3227
10	0.0954	0.1898	0.0063***
11	1.000	0.5630	0.1013
12	0.3061	0.4788	0.0360***
13	0.0783	0.8182	0.0013***
14	0.3009	0.6006	0.0227***
15	0.1320	0.9100	0.3468
16	0.7999	0.4935	0.1844
17	0.9869	0.8913	0.4054
18	0.0111***	0.0992	0.0181***
19	0.0038***	0.0352***	0.0304***
20	0.1064	0.0214***	0.7979

of change in abundance; however, they may suggest changes in species richness.

Results of Additional Analyses

Tables 5 and 6 present results of the tests for trends in data collected prior to plant operation based on Kendall's tau measure of correlation. Results in Tables 5 and 6 suggest that the conclusions drawn from the previous data analysis need to be questioned. Table 5 indicates that a number of species show trends. Table 6 indicates that the differences between control and inpact abundance also show trends for a number of species. Many of the species have a positive trend, while a few exhibit negative trends. A positive trend in our case history indicates either large negative differences that become smaller or positive differences that become larger. A negative trend indicates negative differences becoming larger or positive differences becoming smaller. *If the species with significant trends are eliminated from the BACI analyses, as suggested by Stewart Oaten et al. (1986), then most of the significant differences identified in the initial analysis disappear* (Table 3).

Because imbalancing of sampling data may have resulted in false significances, the BACI analysis was rerun using only the months June, August, and October. These results are presented in Table 7 and, in general, are less significant than the previous analyses. For the west side, only two taxa (6 and 7) are significant ($\alpha = 0.05$). The *p*-values for these taxa are slightly less than 0.05. For the east side, three species are also significant. These are taxa 4, 14, and the unidentified species. The results for taxa 4 and 14 indicate highly significant differences. The combined set of locations indicated four species (4, 6, 14, and 20) were affected. Part of the lack of significance for species in this data may be due to the reduction in sample size. Given the smaller sample sizes in this study, the results, therefore, suggest a change in abundance due to the plant for several species, especially 4 and 14.

To assess the validity of the BACI analysis for this study, an analysis of the before data was done. Here the data from the after period were deleted and the data from the before period were split into two groups. The data before September 1979 were treated as the before group and the data from September 1979 through August 1982 formed the after group. The results are given inTable 8 and provide some interesting observations. By chance alone, one would expect significance one time in 20 tests. The higher the proportion of significant results above this expected level, the less our confidence is in the BACI method as applied to our data. For the combined sites, 4 of 20 species were significant, 2 of 18 were significant for the east site, and 3 of 15 were significant for the west site. Although the species differ, the number that are significant is similar to that of the previous analysis described in Table 5, and the number of effects detected is greater than the number expected due to chance. There could be several explanations for these additional significances. The test may be detecting changes caused by plant construction (not operation), the presence of a sewage treatment plant (started in 1978), or other unknown effects. Alternatively, the significances may reflect the trends in the data at the sites that were detected in Tables 5 and 6.

TABLE 7. *p*-values for the BACI test using the data for the months of June, August, and October. The year 1975 was dropped. The transformation was the same as the one used for the full data (U = untransformed, S = square root, L = log, IS = inverse square root, I = inverse). The sample size is 17 in the before period and 20 in the after period. ***Significant at the 5% level; + + + indicates that no transformation was successful at removing nonadditivity and — indicates that data were zero.

Species	Combined	East	West
1	0.1799 (I)	+ + +	+ + +
2	0.8430 (I)	0.5321 (IS)	0.4929 (I)
3	0.2228 (IS)	0.5222 (I)	0.1435 (I)
4	0.0010 (S)***	0.0028 (S)***	0.0908 (U)
5	0.8192 (IS)	0.5422 (L)	0.3294 (S)
6	0.0027 (I)***	0.1900 (I)	0.0154 (I)***
7	0.0908 (S)	0.1849 (L)	0.0293 (I)***
8	0.5938 (I)	0.5026 (I)	0.2005 (I)
9	0.4461 (I)	0.2861 (I)	0.1244 (I)
10	0.2060 (L)	0.1702 (IS)	0.9878 (L)
11	—	—	—
12	0.1435 (I)	+ + +	0.4020 (S)
13	0.7605 (I)	0.1702 (L)	0.8311 (I)
14	0.0031 (L)***	0.0017 (L)***	0.4929 (U)
15	0.4833 (I)	+ + +	0.1314 (I)
16	0.2931 (I)	0.8789 (I)	0.4553 (U)
17	0.6808 (I)	0.4193 (U)	0.4461 (I)
18	0.2287 (I)	+ + +	0.3219 (I)
19	0.4929 (IS)	0.8549 (L)	0.5123 (IS)
20	0.0109 (IS)***	0.0355 (U)***	0.0530 (S)

Some of the significances associated with the BACI tests may be related to the proportion of zeros in the data. For example, species 8 was present between 24 and 32% of the time in the before period but decreased to almost zero in the after period for all sites except the east impact site where it occurred 15% of the time. Six other species (1, 11, 12, 15, 17, and 18) were absent approximately 70% or more of the time.

Conclusions and Comments

Although a number of fish showed significant change according to the BACI model, there are a number of reasons, both statistical and biological, for doubting whether these changes should be attributed to the plant. A number of species abun-

TABLE 8. *p*-values for the BACI test (Wilcoxon) using the data from the before period only, split into two groups at September 1979. The sample size is 43 for the first square root (ALL = transformation had no effect on the test, U = untransformed, S = square root, L = log, IS = inverse square root, I = inverse). ***Significant at the 5% level; + + + indicates that none of the transformations was successful at removing nonadditivity and — indicates that no test was performed, as most of the data were zeros.

Species	Combined	East	West
1	0.2299 (I)	0.7680 (I)	0.0615 (I)
2	0.1946 (I)	0.1112 (U)	0.6294 (L)
3	0.8801 (I)	0.4508 (I)	0.3883 (I)
4	0.9519 (I)	0.8187 (IS)	0.4841 (IS)
5	0.2230 (IS)	—	0.1616 (IS)
6	0.0036 (IS)***	0.5031 (I)	0.0052 (IS)***
7	0.6294 (S)	0.6208 (IS)	0.9231 (IS)
8	0.0923 (I)	0.0465 (I)***	0.9471 (I)
9	0.2299 (I)	0.8253 (I)	—
10	0.3883 (IS)	0.8944 (I)	0.0161 (S)***
11	0.6950 (ALL)	1.000 (ALL)	—
12	0.4765 (I)	+ + +	—
13	0.2467 (I)	0.8801 (IS)	0.0294 (I)***
14	0.4508 (IS)	0.9856 (I)	0.3405 (I)
15	0.1427 (I)	0.2966 (IS)	0.7860 (I)
16	0.0492 (I)***	0.3623 (I)	0.3051 (S)
17	0.6166 (I)	0.7721 (I)	—
18	0.1477 (I)	0.1427 (IS)	—
19	0.0177 (L)***	0.0741 (IS)	0.1315 (IS)
20	0.0047 (U)***	0.0107 (S)***	0.4051 (I)

dances indicated trends over time in the before period, suggesting interactions that were not included in the model. The sampling program was changed following the initiation of plant operation. Also, the analysis was made more difficult by the large number of zero catches in the data set and the need to transform the data to more closely meet the assumptions of the analysis. Finally, a large number of other factors should be eliminated as possible causative factors before a decision on plant impact is made. Given these other possibilities, we cannot conclusively determine whether the plant has a substantial biological impact on fish populations. It is clear that the results of this analysis would not support a decision against the power company in a court of law.

Our experience with fish data reinforces the commonly held view that fish populations vary considerably in natural settings. While the BACI analysis is intended to be robust for many effects altering natural variance, it is advantageous to have control sites as similar to impact sites as possible. In the case history examined, this is made difficult because the habitat may be changing and this change may cause unwanted interaction if it differs between the control and impact areas. The interaction is suggested by trends in time and suggests that the habitat change in the control site is different from that in the impact site.

The above case study illustrates some of the difficulties in ecological impact assessment. Although the view in the above analyses is rather pessimistic, there are a number of lessons that can aid future studies. Some of these are described below; the papers by Eberhardt and Thomas and their colleagues provide some excellent advice on the design of assessment programs (Thomas et al. 1978; Eberhardt and Thomas 1991; and references therein).

The BACI model is one of the best models for impact assessment, but its success depends on a sound sampling design. Some of the problems encountered (such as the sampling frequency) can be controlled by the agency carrying out the investigation. Control of the sampling program must be maintained throughout the time frame of the study. Major changes should be avoided or at least carefully considered in view of the needs of the proposed analysis. Supplemental information, such as from toxicity tests or on the age structure of the fish population, is relatively inexpensive to collect and can be quite useful for assessing effects and assigning causes (Pratt et al. 1988; Pontasch et al. 1989). Prior information about habitats is needed for selecting sites in the control and impact areas. It is useful to try to anticipate some of the changes that might be caused by other factors (such as construction) and to try to address these factors in the study design. Finally, the role of statistical significance (as opposed to biological significance) should not be overemphasized because statistical significance (with a fixed Type I error rate) is tied to sample size. As the number of sampling times increases (before and after), the amount of change required to produce a significant impact generally decreases. The important game fish, taxa 14, which was one of the taxa showing significant change, had a change in the difference between control and impact of between 1 and 4 fish per catch (i.e. the median difference between control and impact was roughly -1 in the before period and roughly 1 in the after period). Whether this change is important biologically and to what extent the change can be attributed to the plant are difficult questions. A measure of what is biologically significant in assessing impacts and making environmental decisions is important. However, the question of assessing change in field situations is a difficult one that requires sound knowledge not only of the statistical difficulties but also of the biological and political ones. More integration of these components would be beneficial. Sound design requires not only a good statistical model but also an understanding of the underlying biological processes (what to measure) and careful planning (how to measure it well). All of these components are required to achieve defensible impact assessments.

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Fish Species Composition and Lake Abiotic Variables in Relation to the Abundance and Size Structure of Cladoceran Zooplankton

Marco A. Rodríguez,[1] Pierre Magnan, and Sylvain Lacasse

Département de chimie-biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières (Québec) G9A 5H7, Canada

Rodríguez, M. A., P. Magnan, and S. Lacasse. 1993. Fish species composition and lake abiotic variables in relation to the abundance and size structure of cladoceran zooplankton. Can. J. Fish. Aquat. Sci. 50: 638–647.

Redundancy analysis and a multiple regression approach to analysis of variance and analysis of covariance were used to evaluate the influence of fish species composition and abiotic variables on the numerical density, biomass, and mean length of individual cladoceran taxa from 43 Laurentian Shield lakes. Cladoceran assemblage structure was still associated with fish species composition when potentially confounding effects of abiotic variables were accounted for by partial redundancy analysis. Cladoceran abundances were strongly related to the occurrence of white sucker (*Catostomus commersoni*) and lake maximum depth; mean lengths were influenced most by white sucker, water transparency, lake volume, and sampling date. The abundance of bosminids and length of *Holopedium* were strongly related to the occurrence of white sucker even when the influence of abiotic factors was removed by regression. In lakes with white sucker and brook trout (*Salvelinus fontinalis*), *Holopedium* mean length decreased and bosminid abundance increased fourfold relative to lakes with brook trout only; large (>1.2 mm) *Holopedium* abundance was reduced by 76–81%, suggesting that size-selective predation occurred. The increase in bosminid abundance in the presence of white sucker may have been a response to diminished competition by large *Holopedium*, but density compensation was not observed.

Des analyses de redondance et l'approche de la régression multiple pour l'analyse de la variance ainsi que l'analyse de la covariance ont été utilisées pour évaluer l'influence de la composition des espèces de poissons et des variables abiotiques sur la densité, la biomasse et la longueur moyenne de différents cladocères, dans 43 lacs du bouclier laurentien. La structure des communautés de cladocères était associée à la composition des espèces de poissons, même lorsque l'on tenait compte de l'effet potentiellement nuisible de variables abiotiques par l'analyse de redondance partielle. L'abondance des cladocères était fortement liée à la présence du meunier noir (*Catostomus commersoni*) et à la profondeur maximale du lac; les longueurs moyennes étaient principalement influencées par la présence du meunier noir, la transparence de l'eau, le volume du lac et la date d'échantillonnage. L'abondance des bosminidés et la longueur d'*Holopedium* étaient fortement liées à la présence du meunier noir, même lorsque l'effet des facteurs abiotiques était contrôlé par régression. Dans les lacs contenant du meunier noir et de l'omble de fontaine (*Salvelinus fontinalis*), la longueur moyenne d'*Holopedium* diminuait et l'abondance des bosminidés augmentait par un facteur de quatre en comparaison avec les lacs contenant seulement de l'omble de fontaine; l'abondance des grands *Holopedium* (>1,2 mm) était réduite de 76–81 %, suggérant une prédation sélective en fonction de la taille. L'augmentation de l'abondance des bosminidés en présence du meunier noir pourrait être une réponse à une diminution de la compétition par les grands *Holopedium*, mais le phénomène de compensation de la densité n'a pas été observé.

Received April 26, 1991
Accepted September 17, 1992
(JB015)

Reçu le 26 avril 1991
Accepté le 17 septembre 1992

Interpretations of the patterns of distribution and abundance of zooplankton usually focus either on the relationship between species and abiotic factors or on biotic interactions such as competition and predation. In previous studies, explanations based primarily on abiotic factors such as morphometry, physicochemistry, and regional geological history have been applied to patterns of distribution or geographical range (Sprules 1975; Carter et al. 1980), of species relative abundance (Sprules 1977; Yan and Strus 1980; Roff et al. 1981; Pinel-Alloul et al. 1990), and of size structure (Sprules and Holtby 1979; Tessier and Horwitz 1990). Other studies have instead emphasized biotic interactions, mainly predation on zooplankton by invertebrates (Nero and Sprules 1986), by amphibians (Dodson 1970; Morin 1987, and references therein), and by fish (Brooks and Dodson 1965; Galbraith 1967; Nilsson and Pejler 1973; Pope and Carter 1975; Magnan 1988; and many others). Very few studies of zooplankton distribution and abundance (e.g. Dodson 1970; Stenson 1973; O'Brien 1975; Nero and Sprules 1986) have considered the role of biotic and abiotic factors simultaneously. When biotic and abiotic factors are not integrated in the analysis, interpretation of results can be complicated. For example, the putative effects of abiotic factors may be confounded with those resulting from biotic interactions (Nero and Sprules 1986), or may disappear once biotic factors are accounted for (Stenson 1973).

The relationships between various biotic and abiotic factors and observed zooplankton patterns can be evaluated by surveys of many lakes (correlational approach) or by laboratory and

[1]Present address: Département de biologie et des sciences de la santé, Université du Québec à Rimouski, 300, allée des Ursulines, Rimouski (Québec) G5L 3A1, Canada.

TABLE 1. Summary statistics for morphological and physicochemical variables of the 43 study lakes.

Variable	Mean	SD	Minimum	Maximum
Area (ha)	64.07	92.34	3.87	500.78
Volume (m^3)	6 304 824	12 486 800	129 330	59 701 300
Perimeter (m)	5 548	6 354	1 104	35 834
Maximum depth (m)	20.8	11.4	3.9	61.0
Secchi disk transparency (m)	5.91	1.61	3.33	10.96
Conductivity (μS/cm)	23.0	4.7	15.0	39.0
Temperature (°C)	18.6	3.1	13.9	26.9
pH	5.8	0.4	5.1	7.2

field experiments (manipulative approach). The trade-offs between correlational and manipulative methods are well known to ecologists and involve compromises in realism, generality, inferential power, and spatiotemporal resolution (Diamond 1986). Correlational and manipulative studies thus are best viewed as complementary, rather than competing, approaches.

The objective of the present study is to determine whether a key biotic factor, fish species composition, is significantly related to cladoceran abundance and size structure after accounting for among-lake variation in abiotic factors. To this end, we use statistical techniques to analyze survey data on cladoceran abundance and size, fish species composition, and abiotic variables (some of which are seldom amenable to experimental manipulation, e.g. lake morphometry) collected from 43 Laurentian Shield lakes. We focus on cladocerans because fish in the study lakes consume large amounts of cladocerans (Magnan 1988; Tremblay and Magnan 1991), and thus, cladoceran abundance and mean length can be expected to reflect the predation regimes associated with particular fish species compositions.

Materials and Methods

A total of 43 lakes of the Mastigouche Reserve, Québec (46°40′N, 73°20′W), was selected on the basis of fish species composition and lake surface area. Lakes were classified into three categories following Magnan (1988): 15 "brook trout lakes" containing brook trout (*Salvelinus fontinalis*), 14 "creek chub lakes" containing brook trout and creek chub (*Semotilus atromaculatus*), with or without pearl dace (*Margariscus margarita*, formerly *Semotilus margarita*), and 14 "white sucker lakes" containing brook trout and white sucker (*Catostomus commersoni*), with or without pearl dace and creek chub. Gillnet fishing in three of the white sucker lakes indicated that the biomass of creek chub and pearl dace represented less than 1% of the total fish biomass in these lakes. Northern redbelly dace (*Phoxinus eos*) was present in most lakes. Within each of these three categories, lakes were distributed as evenly as possible among five size classes: <10, 10–20, 20–50, 50–100, and >100 ha. Lakes were stratified by surface area to avoid bias due to misrepresentation of particular size classes. All lakes were within a 1500-km^2 area and were exposed to similar climatic conditions. Lake characteristics are summarized in Table 1.

Physicochemistry data and zooplankton samples were collected between 1 and 28 June 1989. Lake area, volume, perimeter, and maximum depth were obtained from (1:20000) bathymetric maps available from the Ministère du Loisirs, de la Chasse et de la Pêche du Québec. A Hydrolab Surveyor II-SVR2 was used to measure epilimnetic water temperature, pH, and conductivity 0.5 m below the water surface at the deepest point of each lake. Water transparency was measured with a 20-cm Secchi disk and an underwater viewer to reduce surface distortion. Fish species composition was coded by means of two dummy binary variables, "chub" and "sucker" (see below). Even though the intensity of fish predation on zooplankton is a continuous quantitative variable that must depend on the relative abundance and size structure of fish species, dummy coding of lakes based on the presence/absence of fish species offers three distinct advantages. First, fish species composition is less variable in time than relative abundance and size structure, which are subject to fluctuations in recruitment, and thus provides a more conservative and stable characterization of lakes. Second, with dummy coding, no prior assumptions about the functional form of the relationship between dependent and independent variables are required (Montgomery and Peck 1982). Third, considerable methodological problems are involved in adequately quantifying the relative abundance and size structure of several fish species in a study that includes a large number of lakes whereas qualitative data on fish species composition are much more readily obtained.

Zooplankton was collected at three stations near the center of each lake by use of a 12-cm Wisconsin plankton net with 80-μm mesh size. Vertical hauls were made from the euphotic zone depth (estimated as twice the Secchi depth; Holmes 1970; Prepas and Trew 1983) to the surface. Whenever the estimated depth of the euphotic zone exceeded the lake depth, hauls were made from 30 cm above the lake bottom, to avoid disturbing the sediments. Samples were preserved in 95% ethanol. All cladocerans in each sample were identified and measured to the nearest 0.01 mm with a dissecting microscope and an ocular micrometer. For each taxon, counts from the three hauls were transformed to densities (organisms per litre) and averaged to obtain mean density for each lake. Cladoceran length was measured from the anterior end of the head to the posterior end of the carapace excluding spines. Biomass was calculated using the length–weight regressions of Bottrell et al. (1976) for *Daphnia*, bosminids, and *Diaphanosoma* and of Rosen (1981) for *Polyphemus* and *Sida*, selecting each time the regression for which the length range was most similar to ours. *Holopedium* weights were calculated using the regression for *Daphnia* because suitable regressions for *Holopedium* (Lawrence et al. 1987; Yan and Mackie 1987) require measurements on unpreserved individuals or several measurements per individual. The means and ranges for *Holopedium* and *Daphnia* carapace length showed considerable overlap in our study (Table 2).

TABLE 2. Summary statistics for numerical density, biomass, and mean length of cladoceran zooplankton. All statistics were calculated across the 43 study lakes. Frequency of occurrence (%) for each taxon is given in parentheses.

Taxon	Mean	SD	Minimum	Maximum
		Numerical density (org/L)		
Bosminids (100)	0.446	0.555	0.015	2.193
Daphnia (100)	0.986	0.914	0.066	4.481
Diaphanosoma (56)	0.033	0.106	0.000	0.674
Holopedium (100)	1.016	1.034	0.008	4.680
Polyphemus (37)	0.007	0.013	0.000	0.053
Sida (44)	0.020	0.076	0.000	0.499
		Biomass (μg/L)		
Bosminids	0.994	1.591	0.012	8.333
Daphnia	4.277	4.344	0.120	19.282
Diaphanosoma	0.064	0.161	0.000	0.870
Holopedium	4.578	5.159	0.007	20.138
Polyphemus	0.062	0.112	0.000	0.379
Sida	0.338	1.207	0.000	7.792
		Mean length (mm)		
Bosminids	0.417	0.067	0.290	0.560
Daphnia	0.902	0.114	0.680	1.150
Diaphanosoma	0.740	0.128	0.510	1.000
Holopedium	0.954	0.209	0.620	1.450
Polyphemus	0.725	0.128	0.470	0.910
Sida	1.407	0.335	0.770	2.410

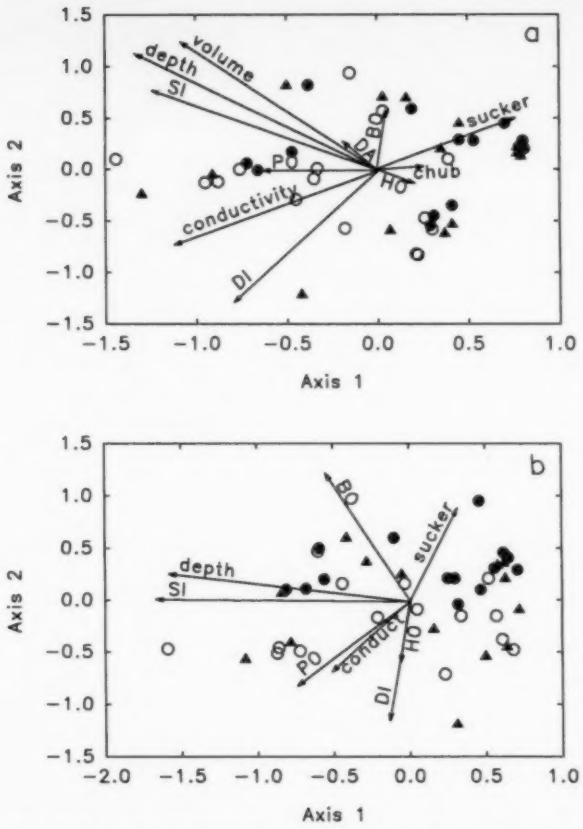

FIG. 1. Ordination biplot of lakes and taxa. An angle near 0° between a taxon arrow and an environmental arrow indicates strong positive correlation of taxon abundance with the environmental factor whereas an angle near 180° indicates strong negative correlation. Environmental and taxon arrows are centered so that the mean value of each transformed variable coincides with the origin of the biplot. (a) Based on numerical density; (b) based on biomass. Open circles represent brook trout lakes; solid triangles represent creek chub lakes; solid circles represent white sucker lakes. BO, bosminids; DA, Diaphnia; DI, *Diaphanosoma*; HO, *Holopedium*; PO, *Polyphemus*; SI, *Sida*.

Individual lakes are the basic sampling unit for statistical tests in this study because counts from three replicate hauls were averaged to obtain means for each lake. The transformation $\ln(X + 1)$ was applied to all environmental variables in Table 1 except pH. The two dummy variables, "chub" and "sucker", were not transformed. Cladoceran variables and environmental variables were transformed whenever necessary to better approximate the regression assumptions of linearity, homoscedasticity, and normality. The transformation $Y = X^{1-b/2}$, where b is derived from the power-law relationship between the mean and the variance (Elliot 1977), was used for the zooplankton data (numerical density and biomass). The residuals were examined graphically to ensure that the regression assumptions were not grossly violated.

Redundancy analysis (CANOCO program, ter Braak 1988), a multivariate technique for direct gradient analysis, was used to determine whether variation in cladoceran abundances was coordinated in response to environmental gradients and to graphically summarize the relationships between cladoceran assemblage structure, fish species composition, and abiotic environmental variables. Variable selection was based on examination of values of the t statistic for the canonical coefficients of individual variables, following a procedure similar to backward elimination in multiple regression (Draper and Smith 1981), with a significance level (alpha) of 0.05. Correspondence analysis (also known as reciprocal averaging, Hill 1973), a weighted-averaging ordination method, was used to evaluate whether the abundance data were curvilinear. The range of the ordination sample scores obtained by correspondence analysis was less than 1.5 standard deviations, indicating that the use of linear methods in this study was appropriate (ter Braak and Prentice 1988). Partial redundancy analysis was used to test whether cladoceran assemblage structure was significantly related to fish species composition after removal of the influence of abiotic covariates (Monte Carlo permutation test for the significance of the sum of all eigenvalues, CANOCO program), following the procedure described by ter Braak (1988).

The response of individual cladoceran taxa to fish species composition and abiotic variables was analyzed by a multiple regression approach to analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Both ANOVA and ANCOVA can be couched in a multiple regression framework when dummy variables are properly coded (Cohen and Cohen 1975; Marascuilo and Levin 1983). ANCOVA increases the power of tests for main effect relative to ANOVA by removing predictable variance associated with covariates from the error term (Tabachnick and Fidell 1989). Nevertheless, when covariates are not significantly related to the dependent variables, the gain in power from decreased error may be offset by the

TABLE 3. Results of redundancy analysis for numerical density and biomass. The significance values for the sum of all eigenvalues (permutational probability estimated by Monte Carlo simulations) are $p = 0.01$ for numerical density and $p = 0.01$ for biomass.

	Numerical density		Biomass	
	Axis 1	Axis 2	Axis 1	Axis 2
Canonical coefficients for standardized variables				
Volume	0.135	0.260		
Max. depth	−0.381	0.012	−0.389	0.088
Conductivity	−0.230	−0.177	−0.112	−0.145
Chub	0.204	0.076		
Sucker	0.178	0.107	0.026	0.207
Correlations of environmental variables with first two axes				
Volume	−0.365	0.503		
Max. depth	−0.448	0.461	−0.647	0.149
Conductivity	−0.375	−0.294	−0.208	−0.402
Chub	0.086	0.017		
Sucker	0.254	0.204	0.129	0.513
Summary statistics for first two axes				
Eigenvalue	0.161	0.104	0.170	0.076
Fraction of variance explained	0.540	0.348	0.647	0.289
Species–environment correlation	0.673	0.656	0.675	0.646

loss of degrees of freedom due to inclusion of the covariates (Tabachnick and Fidell 1989). Therefore, instead of forcing all measured abiotic variables into the model as covariates, we used multiple regression to select a subset of covariates that were predictably associated with the dependent variables, as prescribed by Marascuilo and Levin (1983).

In addition to the physicochemical and morphological variables, sampling date was included as a covariate to account for possible seasonal trends during the sampling period (Draper and Smith 1981). Stepwise regression and backward elimination (Draper and Smith 1981) were used for selection of abiotic variables, with the level for entry or removal of individual variables set at alpha = 0.05 (SYSTAT, Wilkinson 1988). In most cases the same regression equation was obtained by both approaches, but in case of disagreement, preference was given to the equation with the highest adjusted R^2. Among the transformed abiotic variables, pairwise (bivariate) correlations greater than 0.60 in absolute value occurred only among the morphometric variables (volume, area, maximum depth, and perimeter; $r = 0.66$–0.96) and between sampling date and epilimnetic water temperature ($r = 0.81$). Instead of arbitrarily excluding one or several of these variables on the basis of pairwise correlations, with potential loss of information (James and McCulloch 1990), all abiotic variables were included in the regressions. After variable selection was completed, models were screened for multicollinearity by examination of the tolerance of individual variables (Tabachnick and Fidell 1989). Pairs or triplets of morphometric variables with tolerances less than 0.20 appeared in some models. When this occurred, the variables with low tolerance were dropped one at a time, and parameters were recalculated to yield alternative reduced models. If necessary, this procedure was repeated until no tolerances were less than 0.20. The regression with the highest adjusted R^2 was then selected as the final regression equation. Tolerances of individual variables were greater than 0.72 in all final equations.

The hypothesis of no differences among lake types (no significant main effect associated with fish species composition) was tested by ANCOVA if reliable abiotic predictors were found and by ANOVA if no abiotic variables were reliable predictors. The model used for ANOVA/ANCOVA analyses (Montgomery and Peck 1982) is

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \sum^{k} \alpha_i C_i + \sum^{k} \delta_{i1} X_1 C_i + \sum^{k} \delta_{i2} X_2 C_i + \epsilon$$

where Y is the dependent variable and ϵ is an error term. X_1 ("chub") and X_2 ("sucker") are dummy variables coding for lake type as follows:

	Coding	
Lake type	X_1	X_2
Trout	0	0
Chub	1	0
Sucker	0	1

The C_i are the abiotic covariates and k is the number of covariates in the model. In the ANOVA model (without abiotic covariates: $k = 0$) the intercept β_0 equals the mean value of the dependent variable Y for brook trout lakes. The coefficient β_1 is the difference in means between creek chub lakes and brook trout lakes and can be interpreted as the effect of adding creek chub to brook trout lakes whereas β_2 is the difference in means between white sucker lakes and brook trout lakes and can be interpreted as the effect of adding white sucker to brook trout lakes. A similar interpretation of regression coefficients

applies in the ANCOVA model: β_0 is the dependent variable mean for brook trout lakes adjusted for the abiotic covariates, β_1 measures the change in adjusted means resulting from addition of white sucker to brook trout lakes.

Testing whether no differences exist among lake types is equivalent to testing whether β_1 and β_2 are simultaneously equal to zero when the model includes the intercept and all other predictor terms (Montgomery and Peck 1982). We calculated significance values for the null hypothesis of no effect of fish species composition ($\beta_1 = \beta_2 = 0$) as well as for the hypotheses of no effect of creek chub addition ($\beta_1 = 0$) and of no effect of white sucker addition ($\beta_2 = 0$). Tests were conducted for the pooled within-group relationship between the abiotic covariates and the dependent variables (F-tests for the α_i coefficients above) by allowing the abiotic variables to enter after forcing the two dummy variables into the model (Marascuilo and Levin 1983). We also tested for the presence of lake type by covariate interactions (F-tests for the δ_{i1} and δ_{i2} coefficients above) following Marascuilo and Levin (1983); nonsignificant interaction terms were dropped before calculation of coefficients in the final model. Before each ANOVA or ANCOVA test, we verified the homogeneity of variance of dependent variables (Bartlett's test). Differences in abiotic variable means among lake types were not significant (ANOVAs for individual abiotic variables: all $p \geq 0.22$; multivariate ANOVA for all abiotic variables combined: $p = 0.664$). Multiple coefficients of determination (R^2) were used to quantify the fraction of variance explained by the ANOVA and ANCOVA models.

Results

More than 30 000 cladocerans were sampled in the 43 lakes, and 99.9% of these individuals were accounted for by six taxa: bosminids, *Daphnia*, *Diaphanosoma*, *Holopedium gibberum*, *Polyphemus pediculus*, and *Sida crystallina* (Table 2). The cladocerans *Leptodora kindtii*, *Chydorus*, *Acroperus*, and *Ceriodaphnia*, as well as *Chaoborus* larvae and water mites, were found in some lakes but were not included in the analyses because their density and frequency of occurrence were very low. Results from a regional limnological survey (Bourassa et al. 1972) suggest that in these lakes the most abundant species in the genus *Daphnia* is *D. catawba*, with four or five more species occasionally present at low abundances. Three bosminids, *Bosmina longirostris*, *Eubosmina longispina*, and *E. coregonis*, are found in the study area. Regionally, *Diaphanosoma birgei* and *Ceriodaphnia lacustris* seem to be the sole representatives of their respective genera (Bourassa et al. 1972). Aggregation of species into higher categories as used here might reduce the likelihood of detecting (true) effects of environmental variables, especially if these effects are weak, but aggregation should not increase the likelihood of erroneously identifying (accepting as true) an inexistent effect.

The results of redundancy analysis for numerical density and biomass were used to generate ordination biplots (Fig. 1) that simultaneously represent the similarities between lakes based on their cladoceran assemblages and the relationships between the abundance (numerical density and biomass) of cladoceran taxa and the environmental data (fish species composition and abiotic variables). The fraction of variance explained by the ordination taxa (Table 3) represents the fraction of the variance in all taxa–environment covariances that is accounted for by the axes (ter Braak 1987). The fraction of the variance jointly explained by the first two axes is high, both for numerical density ($V_{exp} = 0.89$) (Fig. 1a) and for biomass ($V_{exp} = 0.94$) (Fig. 1b). Therefore, not much information is lost by discarding the third and lower axes.

Taxa arrows point in different directions, with very little alignment between arrows (Fig. 1), which indicates that individual taxa differed from each other in their response to environmental variables. The angles between environmental and taxa arrows and the relative length of the arrows can be used jointly to infer the direction of taxa responses to environmental variables (ter Braak 1987).

The ordinations indicate that assemblage response to environmental gradients was relatively continuous, as the lakes could not be unambiguously assigned to discrete subgroups on the basis of assemblage structure (Fig. 1). The biplots based on numerical density and biomass resemble each other. The environmental variables most strongly related to assemblage structure are maximum depth, conductivity, the occurrence of white sucker, and, in the case of numerical density, lake volume, which is highly correlated ($r = 0.85$) with maximum depth. The occurrence of creek chub is only weakly related to numerical density and is unrelated to biomass. The biplots show that in deep lakes the numerical density and biomass of *Sida*, *Daphnia*, and *Polyphemus* are relatively high. In low-conductivity lakes with white sucker the numerical density and biomass of *Polyphemus* and *Diaphanosoma* are relatively low, as is the biomass of *Holopedium*.

Redundancy analysis showed a statistically significant relationship between cladoceran assemblage structure and the ensemble of variables describing fish species composition and abiotic features (Table 3). Partial redundancy analysis revealed that cladoceran assemblage structure was still significantly related to fish species composition after removal of the influence of abiotic variables (Monte Carlo probabilities based on 1000 permutations: $p < 0.05$ for numerical density; $p < 0.01$ for biomass).

The small eigenvalues and taxa–environment correlations (Table 3) indicate that the overall relationship between assemblage structure and environmental variables was not very strong and that only a small portion of the overall variance in assemblage structure was accounted for by the environmental variables. The ordinations assume that all taxa react to the same composite gradients of environmental variables (ter Braak and Prentice 1988). However, the biplots show that taxa responses to environmental variables were individualistic rather than coordinated because each taxon responded to different combinations of environmental variables. In contrast with ordination, in multiple regression, separate composite gradients can be constructed for each taxon, thus allowing for more detailed descriptions of taxa responses to environmental gradients and more accurate prediction than ordination (ter Braak and Prentice 1988).

Separate analyses of the responses of individual taxa by a multiple regression approach to ANOVA and ANCOVA (Table 4) account for only a small (<26%) or moderate (39–70%) fraction of the variance in the dependent variables. Simple bivariate regression yielded results generally similar to those obtained by multiple regression (Table 4), suggesting that the multivariate data structure was relatively simple. In general, few abiotic variables entered the final models (Table 4). The limited predictive power of most of the abiotic variables may be partly due to the regional nature of the study because natural variation in physicochemical variables is relatively low in the study area (Table 1).

TABLE 4. Summary of ANOVA/ANCOVA analyses for individual taxa. FSC: main effect associated with fish species composition. Abiotic covariates in ANCOVAs are in italics. Standardized regression coefficients (Montgomery and Peck 1982) are presented to adjust for arbitrary differences in measurement scales between independent variables. Asterisks accompanying an independent variable in the ANOVA/ANCOVA models indicate that the variable was significantly related to the dependent variable in a simple bivariate regression. For these variables, the sign of the regression coefficient was always the same in bivariate regression and in the ANOVA/ANCOVA model $*p < 0.05$; $**p < 0.01$.

	Numerical density				Biomass				Mean length			
Taxon	Effect or variables	Std. coeff.	p	R^2	Effect or variables	Std. coeff.	p	R^2	Effect or variables	Std. coeff.	p	R^2
Bosminids	FSC		0.00020		FSC		0.00040		FSC		0.18175	
	Sucker**	0.586	0.00017		Sucker**	0.487	0.00084		Sucker	0.018	0.88413	
	Chub	0.030	0.82981		Chub	−0.060	0.65621		Chub	−0.190	0.12107	
	Max. depth	0.347	0.00783		*Max. depth***	0.495	0.00016		*Transp.***	0.530	0.00007	
				0.410				0.460	*Volume***	0.348	0.00549	
												0.585
Daphnia	FSC		0.85016		FSC		0.70694		FSC		0.69109	
	Sucker	−0.090	0.60055		Sucker	−0.147	0.41756		Sucker	−0.152	0.40122	
	Chub	−0.075	0.66026		Chub	−0.044	0.80604		Chub	−0.048	0.78972	
	*Max. depth**	0.367	0.01868					0.017				0.018
				0.142								
Diaphanosoma	FSC		0.08859		FSC		0.11557		FSC		0.69803	
	Sucker	−0.297	0.06917		Sucker	−0.293	0.07213		Sucker	−0.075	0.73698	
	Chub	−0.320	0.04969		Chub	−0.288	0.07621		Chub	0.141	0.53084	
	*Conduct.***	0.387	0.00860		*Conduct.***	0.400	0.00668					0.032
				0.254				0.255				
Holopedium	FSC		0.28636		FSC		0.09913		FSC		0.00006	
	Sucker	−0.038	0.82733		Sucker*	−0.289	0.09810		Sucker**	−0.501	0.00002	
	Chub	0.225	0.20526		Chub	0.073	0.66901		Chub	−0.312	0.00400	
				0.061				0.109	Date**	0.625	<0.00001	
									Transp.	0.286	0.00269	
												0.703
Polyphemus	FSC		0.22258		FSC		0.20620		FSC		0.98670	
	Sucker	−0.219	0.21418		Sucker	−0.235	0.18302		Sucker	0.038	0.89727	
	Chub	0.082	0.63877		Chub	0.069	0.69107		Chub	−0.011	0.96931	
				0.072				0.076				0.002
Sida	FSC		0.15634		FSC		0.12298		FSC		0.07015	
	Sucker	−0.190	0.18964		Sucker	−0.205	0.15910		Sucker	−0.487	0.03425	
	Chub	−0.273	0.06298		Chub	−0.292	0.04856		Chub	−0.354	0.10180	
	*Max. depth***	0.589	0.00003		*Max. depth***	0.580	0.00004		*Perimeter**	0.587	0.00896	
				0.393				0.390				0.462

Maximum depth showed a positive relationship with the abundance of bosminids, *Daphnia*, and *Sida*. The addition of white sucker was related to increases in bosminid abundance. Conductivity was positively related to the abundance of *Diaphanosoma*. Bosminid mean length increased with Secchi disk transparency and with lake volume. The mean length of *Holopedium* decreased with the addition of white sucker or creek chub and increased with Secchi disk transparency and sampling date. *Sida* mean length appeared to be related to lake perimeter, but this interpretation is complicated by the presence of significant interactions between lake perimeter and the two dummy variables in this model.

ANOVA/ANCOVA results closely agree with those obtained by redundancy analysis (cf. Fig. 1) and indicate that the addition of white sucker and abiotic variables, in particular lake maximum depth, are significantly related to cladoceran abundance and mean length.

Although generally the R^2 values for the ANOVA and ANCOVA models are not high, the magnitude of changes associated with the addition of white sucker is large. Bosminid abundance in white sucker lakes was approximately four times higher than in brook trout lakes; numerical density was 0.19 org/L (SE = 0.040) in brook trout lakes and 0.82 org/L (SE = 0.177) in white sucker lakes, an increase of 337%; biomass was 0.50 μg/L (SE = 0.137) in brook trout lakes and 1.85 μg/L (SE = 0.610) in white sucker lakes, an increase of 273%. The increase in abundance was distributed more or less evenly among size classes (Fig. 2a) and, as a consequence, the mean length of bosminids was not affected by the addition of white sucker (Table 4). In contrast, the decline in mean length of *Holopedium* in white sucker lakes relative to brook trout lakes was caused by reductions in the abundance of individuals larger than 1.1 mm and by increases in the abundance of individuals smaller than 1.1 mm (Fig. 2b). Mean length of *Holopedium* was 0.80 mm (SE = 0.035) in white sucker lakes, 0.99 mm (SE = 0.061) in creek chub lakes, and 1.06 mm (SE = 0.045) in brook trout lakes.

Because the size range of *Holopedium* was large (Table 2), the variation in *Holopedium* size structure in relation to environmental variables (particularly with respect to possible size-dependent predation in white sucker lakes) was examined more closely by analyses of the numerical density of each of seven size classes (2-mm intervals) (Table 5). The abundance of small *Holopedium* (<0.8 mm) was negatively related to sampling date and Secchi disk transparency whereas the abundance of larger individuals (1.4–1.6 mm) was positively related to temperature. Large individuals (≈1.2–1.6 mm) were reduced in white sucker lakes, and the magnitude of this reduction was

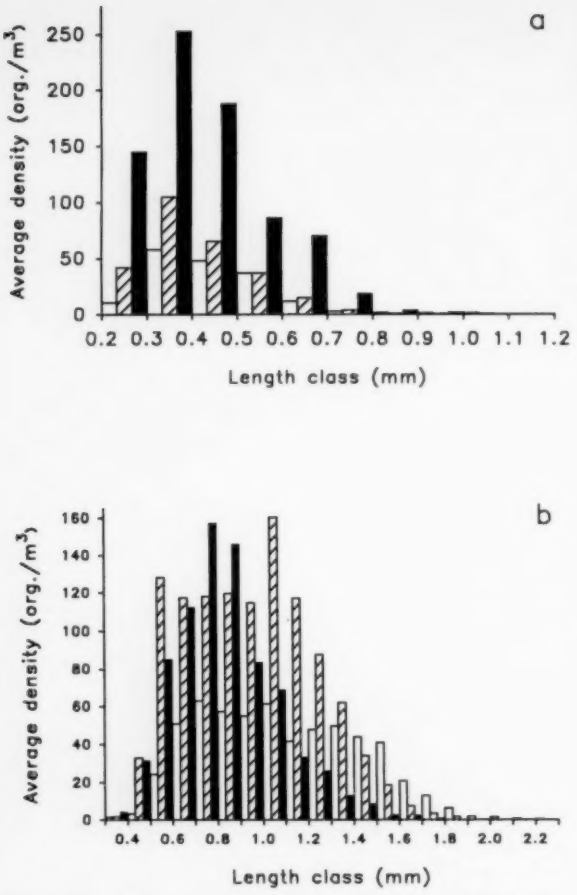

FIG. 2. Length–frequency distributions of bosminids and *Holopedium* in relation to fish species composition. (a) Bosminids; (b) *Holopedium*. Open bars represent brook trout lakes; hatched bars represent creek chub lakes; solid bars represent white sucker lakes. The standard error/mean ratio for transformed data is 0.17 for bosminids and 0.15 for *Holopedium* (median value across size classes).

large. For individuals larger than 1.2 mm, numerical density declined from 0.23 org/L (SE = 0.072) in brook trout lakes to 0.06 org/L (SE = 0.025) in white sucker lakes (76% reduction), and biomass declined from 2.99 μg/L (SE = 0.964) in brook trout lakes to 0.57 μg/L (SE = 0.247) in white sucker lakes (81% reduction). Small *Holopedium* (<1.1 mm) in white sucker lakes increased relative to brook trout lakes (Fig. 2b), but this increase was not statistically significant (Table 5), presumably because within-group variances were large.

Discussion

This study extends earlier work (Brooks and Dodson 1965; Nilsson and Pejler 1973; Pope and Carter 1975; Magnan 1988) by providing evidence that variations in the abundance and size structure of cladoceran zooplankton are still associated with fish species composition when potentially confounding effects of abiotic variables are accounted for. More specifically, we show that the addition of white sucker is associated with marked changes in the abundance and size structure of bosminids and

TABLE 5. Summary of ANOVA/ANCOVA analyses for *Holopedium* length classes. The dependent variable in all cases is numerical density. FSC: main effect associated with fish species composition. Abiotic covariates in ANCOVAs are in italics. Standardized regression coefficients (Montgomery and Peck 1982) are presented to adjust for arbitrary differences in measurement scales between independent variables. Asterisks accompanying an independent variable in the ANOVA/ANCOVA models indicate that the variable was significantly related to the dependent variable in a simple bivariate regression. For these variables, the sign of the regression coefficient was always the same in bivariate regression and in the ANOVA/ANCOVA model. $*p < 0.05$; $**p < 0.005$.

Length class (mm)	Effect or variables	Std. coeff.	p	R^2
<0.60	FSC		0.57459	
	Sucker	0.090	0.52413	
	Chub	0.148	0.30390	
	*Date***	−0.588	0.00004	
	*Transp.**	−0.328	0.01095	
				0.438
0.6–0.79	FSC		0.08407	
	Sucker	0.216	0.15769	
	Chub	0.340	0.03021	
	*Date***	−0.538	0.00030	
				0.338
0.8–0.99	FSC		0.83978	
	Sucker	0.045	0.80153	
	Chub	0.106	0.55782	
				0.009
1.0–1.19	FSC		0.17738	
	Sucker	−0.182	0.29828	
	Chub	0.152	0.38593	
				0.083
1.2–1.39	FSC		0.03711	
	Sucker*	−0.324	0.05874	
	Chub	0.111	0.50696	
				0.152
1.4–1.59	FSC		0.00013	
	Sucker**	−0.590	0.00006	
	Chub	−0.089	0.48882	
	*Temperature**	0.380	0.00609	
	Perimeter	0.314	0.01767	
				0.528
>1.59	FSC		0.06764	
	Sucker*	−0.394	0.02470	
	Chub	−0.107	0.53072	
				0.126

Holopedium (cf. Magnan 1988) and that this result still holds when the influence of lake morphometry, physicochemistry, and sampling date is removed by regression.

The influence of white sucker on *Holopedium* appears to be twofold. First, white sucker may have a direct impact on *Holopedium* populations because small (≈1.3–2.3 cm) individuals are planktivorous (Carlander 1969; Scott and Crossman 1973), and larger individuals consume considerable amounts of cladocerans, especially late in the summer (Tremblay and Magnan 1991). A second potential impact is indirect and is mediated by competition between white sucker and brook trout. In white sucker lakes, the diet of brook trout shifts strongly from benthic invertebrates to cladocerans (primarily *Holopedium* and *Daphnia*), which can be the predominant food item for brook

trout during the summer (Magnan 1988; Tremblay and Magnan 1991).

The addition of creek chub was associated with changes in the mean size of *Holopedium*, but not with changes in abundance or size structure of bosminids (Table 4). This contrasts with previous work (Magnan 1988) that showed that the abundance and mean size of bosminids, as well as the mean size of *Holopedium*, were related to the addition of creek chub, although the impact of creek chub was weaker than that of white sucker. Zooplankton samples were collected later in the summer (15–19 July) in Magnan's (1988) study than in the present study (1–28 June), and the difference in the results of the two studies might be explained by seasonal differences in the strength of competition between creek chub and brook trout. Creek chub and brook trout feed on benthic invertebrates, a resource that becomes progressively scarcer in these lakes during the summer (Magnan and FitzGerald 1982; Tremblay and Magnan 1991). Competition between creek chub and brook trout for benthic food is stronger when food is scarce, and therefore, brook trout feed more intensely on zooplankton and less on benthos as the summer advances (Magnan and FitzGerald 1982). If the shift of brook trout to zooplankton indirectly benefits bosminids (e.g. by reducing predators or competitors; see below), this may explain why bosminid abundance and size were related to the occurrence of creek chub in July but not in June.

Among the abiotic variables, measures of lake size (volume, perimeter, and particularly maximum depth) were useful covariates of cladoceran abundance and mean size. The abundance of three taxa, bosminids, *Daphnia*, and *Sida*, increased with lake maximum depth, possibly in response to a relative decrease in the intensity of competition, predation, or both in deeper lakes (Tessier and Horwitz 1990, and references therein). Resource heterogeneity and the availability of refugia increase with lake depth, thereby enabling competing species to reduce their overlap by resource partitioning along the vertical dimension and also allowing prey migration to depths where predators are restricted by factors such as temperature, oxygen concentration, or visibility. Stratification, a form of vertical heterogeneity, has been previously related to zooplankton size structure, resource partitioning, and refuge from predation (Wright and Shapiro 1990; Tessier and Horwitz 1990; Tessier and Welser 1991). The increase in bosminid mean length with lake volume also may reflect the influence of maximum depth because maximum depth and volume are highly correlated.

Interpretation of the increase in mean length of *Holopedium* with sampling date (Table 4) is complicated by the strong correlation between sampling date and temperature because the potential effects of these two factors cannot be disentangled without additional data. It is possible that the foraging activity of planktivorous fish, which preferentially consume large *Holopedium*, decreases with temperature and therefore that large *Holopedium* are more abundant late in June, when lake temperatures are highest (Table 5). The range of temperatures measured in the study lakes (13.9–26.9°C at 0.5 m depth, Table 1) is certainly broad enough to allow for thermal limitation of the activity of brook trout, which have a fundamental thermal niche (Christie and Regier 1988) of 13–17°C (Lacasse and Magnan 1992). A more parsimonious explanation for the increase in mean length of *Holopedium* with sampling date is growth of individuals over time.

The absence of statistical relationships between *Daphnia* and fish species composition (Magnan 1988; this study, Table 4) is somewhat puzzling. In the study lakes, *Daphnia* resembles *Holopedium* in abundance and carapace size (Table 2), yet apparently, *Holopedium* responded to increased predation by fish in white sucker lakes, while *Daphnia* did not. *Daphnia* and *Holopedium* may differ in their vulnerability to fish predators because of differences in visibility or behavior. Differences in visibility between the two taxa do not depend solely on carapace size because in *Holopedium* the carapace, which is the most visible part of the body, is surrounded by a thick, transparent mucilage envelope. Hence, at comparable carapace lengths the body (carapace plus mucilage) is smaller, and possibly the visibility is less, in *Daphnia* than in *Holopedium*. However, other factors that affect visibility, such as the size and number of eggs, eye size, gut contents, body pigmentation, and motion (Zaret 1980), can also affect the relative vulnerability of the two taxa to predation. Motion-based differences in visibility between the two taxa probably exist because *Daphnia* swims in a characteristic "hop-and-sink" pattern whereas *Holopedium* glides on its back (Margalef 1983). Behavioral differences between *Daphnia* and *Holopedium* may also contribute to the different responses of the two taxa to the addition of white sucker. Although patterns of vertical migration for *Holopedium* are quite variable (Schindler and Novén 1971; Yan and Larsson 1988), opposite patterns of vertical migration have been reported for *Daphnia* and *Holopedium* in small lakes of northeastern Pennsylvania (Tessier 1986) and the Laurentian Shield, Quebec (Naud and Magnan 1988), with *Daphnia* rising at dusk and sinking at dawn (nocturnal vertical migration) and *Holopedium* rising at dawn and sinking at dusk (reverse vertical migration). In lakes where this pattern applies, *Holopedium* is more likely than *Daphnia* to be exposed to daytime predation by brook trout, which are visual feeders.

The decrease in mean length of *Holopedium* in white sucker lakes relative to brook trout lakes suggests that the abundance of large *Holopedium* was reduced by size-selective predation. Reductions in the abundance and mean size of *Holopedium* in response to fish predation have been reported previously by Stenson (1973) for Swedish lakes and by O'Brien (1975) for Alaskan ponds. In white sucker lakes, increases in bosminid abundance accompanied the decrease in biomass and mean length of *Holopedium*. Decreases in the abundance and mean size of large grazing cladocerans, usually daphnids, and concurrent increases in small cladoceran (e.g. *Bosmina* and *Ceriodaphnia*) abundance are common responses to increased predation by fish (Hrbáček 1962; Brooks and Dodson 1965; Galbraith 1967; Sprules 1972; Mills and Forney 1983; Vanni 1986; Post and McQueen 1987), and stimulation of bosminids upon removal of large cladoceran grazers by fish has been suggested to occur through decreased competition for flagellates (Kerfoot 1987).

If bosminids and large *Holopedium* compete, a negative relationship between the abundances of the two taxa (density or biomass compensation) should exist at equilibrium, provided that samples are collected from a number of relatively homogeneous localities (Schoener 1974; Hallet and Pimm 1979). However, evidence for density compensation is weak in scatterplots of bosminid abundance versus large (>1.2 mm) *Holopedium* abundance (Fig. 3). The plots clearly show that in white sucker lakes, bosminid abundance increases and the abundance of large *Holopedium* decreases; nevertheless, there is no significant linear relationship between the abundances of the two taxa after accounting for the addition of white sucker or after

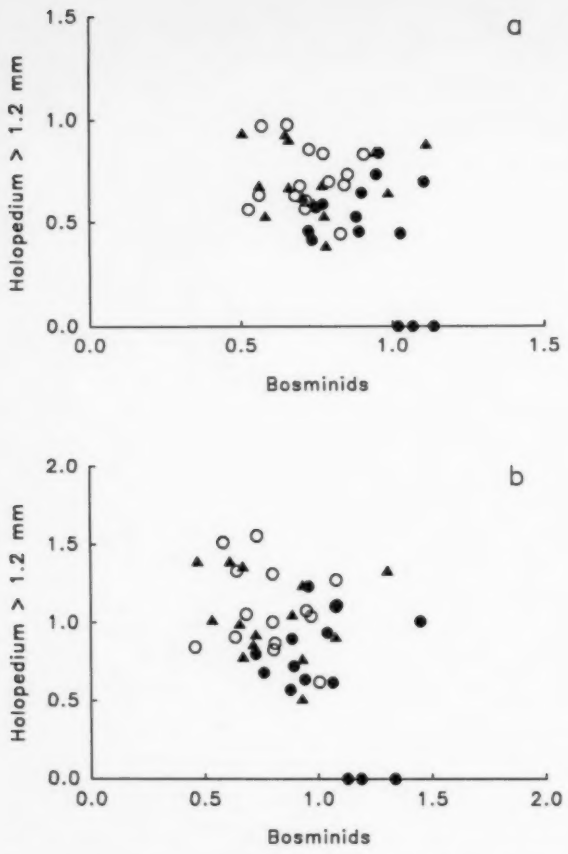

FIG. 3. Abundance of bosminids versus abundance of large (>1.2 mm) *Holopedium*. The transformation $Y = X^{1-b/2}$ (Elliot 1977) was applied to the density and biomass of both taxa. (a) Numerical density; (b) biomass. Open circles represent brook trout lakes; solid triangles represent creek chub lakes; solid circles represent white sucker lakes.

deletion of lakes without *Holopedium*. The Pearson correlation between the numerical density of bosminids and large *Holopedium* is -0.42 ($p < 0.01$), and the partial correlation between these same variables with the effect of white sucker held constant is -0.20 (not significant). When the three lakes without large *Holopedium* are dropped, the Pearson correlation is -0.07 (not significant), and the partial correlation is 0.08 (not significant). The Pearson correlation between the biomass of bosminids and large *Holopedium* is -0.39 ($p < 0.01$), and the partial correlation between these same variables with the effect of white sucker held constant is -0.20 (not significant). When the three lakes without large *Holopedium* are dropped, the Pearson correlation is -0.16 (not significant), and the partial correlation is 0.02 (not significant). A possible explanation for the apparent absence of density compensation is that the effects of competition were masked by environmental "noise", i.e. by factors that affected the abundance of bosminids and large *Holopedium* but that did not systematically differ between white sucker lakes and brook trout lakes. Yan et al. (1988) have shown that although abundance patterns consistent with competitive reduction of *Holopedium* by *Daphnia* in Canadian Shield lakes may not be evident in synoptic surveys, such patterns are detectable in lakes that are intensively monitored.

The observed differences in bosminid and *Holopedium* abundances between white sucker lakes and brook trout lakes are consistent with explanations based on competition (e.g. Kerfoot 1987). Nevertheless, these differences could be interpreted equally well in terms of processes unrelated to competition, such as changes in predation by invertebrate zooplankton (O'Brien 1975) or in food levels for grazers. For example, in white sucker lakes, chaoborid larvae are consumed in large quantities by brook trout and white sucker (Tremblay and Magnan 1991). Thus, in these lakes the observed increases in bosminid abundance could be a response to decreased predation on bosminids by chaoborid larvae rather than to release from competition with large *Holopedium*.

Acknowledgments

We thank D. Courtois, J.-F. Duchesne, E. Harnois, S. Gour, and L. Thiffeault for their field and laboratory assistance and J. Archambault, C. Garceau, and M. Bellemare from the ministère du Loisir, de la Chasse et de la Pêche du Québec for logistic support. We benefited greatly from the comments of anonymous reviewers. Participants at the first symposium of the Groupe de recherche interuniversitaire en limnologie et en environnement aquatique (GRIL) provided useful feedback. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and le Fonds de formation des chercheurs et d'action concertée du Québec to P. Magnan. S. Lacasse was supported by an NSERC postgraduate fellowship.

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Fatty Acid Composition of Lipids in the Eggs and Alevins from Wild and Cultured Chinook Salmon (*Oncorhynchus tshawytscha*)

H. J. Ashton, D. O. Farkvam, and B. E. March

Department of Animal Science, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada

Ashton, H. J., D. O. Farkvam, and B. E. March. 1993. Fatty acid composition of lipids in the eggs and alevins from wild and cultured chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 50: 648–655.

The fatty acid profiles of lipids extracted from eggs and alevins of cultured chinook salmon (*Oncorhynchus tshawytscha*) were significantly different than those from eggs and alevins of wild fish of the same stock. The mean total concentrations of *n*3 fatty acids in the total lipid of eggs from wild and cultured broodstock were 40.2 and 29.4% and in the polar lipid 45.5 and 38.7% respectively. The mean concentrations of *n*3 fatty acids in the total lipid of alevins from wild and cultured broodstock were 35.4% and 23.2% and in the polar lipid 43.0 and 38.2%, respectively. The ratios of *n*3:*n*6 fatty acids were lower in both the neutral and polar lipids of eggs and of alevins from the cultured fish than in those of wild fish. The higher *n*3:*n*6 fatty acid ratios in the eggs and progeny of wild fish reflected the higher ratios in lipids of components of the natural marine food chain.

Les profils des acides gras des lipides extraits d'oeufs et d'alevins de saumon quinnat (*Oncorhynchus tshawytscha*) d'élevage différaient de façon significative de ceux obtenus à partir de poissons sauvages du même stock. Les concentrations moyennes totales d'acides gras *n*3 des lipides totaux des oeufs de géniteurs sauvages et des géniteurs d'élevage étaient, respectivement, de 40,2 et 29,4 % et celles des lipides polaires de 45,5 et 38,7 %. Les concentrations moyennes d'acides gras *n*3 dans les lipides totaux des alevins d'origine sauvage ou d'élevage étaient, respectivement, de 35,4 et 23,2 %, tandis qu'elles étaient respectivement de 43,0 et 38,2 % dans les lipides polaires. Les rapports entre les acides gras *n*3 et *n*6 étaient moins élevés dans les lipides neutres et polaires des oeufs et des alevins des poissons d'élevage que dans ceux des poissons sauvages. Les rapports plus élevés acides gras *n*3 : acides gras *n*6 notés chez les oeufs et la progéniture des poissons sauvages reflètent les rapports plus élevés des lipides de la chaîne alimentaire marine naturelle.

Received March 9, 1992
Accepted August 26, 1992
(JB431)

Reçu le 9 mars 1992
Accepté le 26 août 1992

Fatty acids with the *n*3 configuration are essential for the normal development of salmonid fish. A deficiency of *n*3 fatty acids in the broodstock diet decreases the number and size of eggs produced (Watanabe et al. 1984), increases the incidence of early embryonic mortality, and causes physiological dysfunction of the developing fish (Watanabe 1982; Leray et al. 1985). The diet of the broodfish is the only source of essential fatty acids for egg formation, and hence for embryonic development. The present study was conducted to determine whether there are differences between wild and cultured stocks of chinook salmon (*Oncorhynchus tshawytscha*) in the lipid composition of their eggs and alevins, attributable to differences in the fatty acid composition of their diets.

Materials and Methods

Experiment 1: Comparison of Lipid Composition and Fatty Acid Profiles of Eggs of Two Stocks of Cultured and Wild Chinook Salmon

Broodstock

The cultured broodstock were 3-yr-old fish of Big Qualicum (BQ) and Robertson Creek (RC) parentage, from the broodstock rearing site of Sea Spring Salmon Farm Ltd. in Genoa Bay, Vancouver Island. The fish of each stock were fed two different formulated diets (Diets 1 and 2, Table 1) until they were transferred to freshwater, 2–4 wk prior to spawning. They were not fed subsequent to transfer.

Eggs from wild BQ and RC stocks of chinook salmon returning to their natal streams to spawn were sampled at the Big Qualicum and Robertson Creek hatcheries operated by the Department of Fisheries and Oceans on Vancouver Island. In order to match the eggs of wild and cultured broodstock, sampling at each hatchery was conducted when the peak returns of 3-yr-old fish were known to occur. Only those fish falling within the size range of 3-yr-old fish were sampled. Scales were taken coincidentally with egg samples for confirmation of age.

Egg collection and sampling

Five females were randomly selected from each group except for the RC stock fed Diet 2. Only three fish in this group matured, all of which were sampled. Samples (30–60 g) of the eggs from individual fish were taken within 10 min of stripping, and before fertilization, for determination of the lipid composition and fatty acid profile. The eggs were drained of their ovarian fluid, placed in plastic bags, and immediately frozen. The egg samples were repackaged, without thawing, into oxygen barrier bags (W. Grace and Co. Canada Ltd., Mississauga, Ont.) and vacuum-sealed within a week of collection for storage at −35°C. Samples were analyzed within 10 mo of collection.

The logistics of sampling at the commercial and two gov-

TABLE 1. Lipid concentrations and fatty acid profiles of the lipid contained in formulated diets fed to cultured broodstock. Diets 1 and 2 were fed in Experiment 1 and Diet 3 in Experiment 2.

Fatty acid	Diet 1, %	Diet 2, %	Diet 3, %
14:0	5.59	5.37	5.70
14:1*n*9	0.51	0.34	
16:0	17.00	16.75	16.97
16:1*n*9	0.24	0.00	
16:1*n*7	5.07	5.75	6.50
16:1*n*5	0.24	0.00	
18:0	3.14	2.76	2.93
18:1*n*9	17.08	19.40	16.76
18:1*n*7	2.77	3.09	2.59
18:1*n*5	0.54	0.35	0.48
18:2*n*6	3.42	5.03	5.90
18:3*n*6	0.33	0.00	
18:3*n*3	0.79	0.99	1.53
18:4*n*3	1.52	1.03	1.64
20:1*n*11	4.28	2.70	5.79
20:1*n*9	2.76	5.78	
20:1*n*7	0.36	0.28	
20:4*n*6	0.48	0.44	0.67
20:4*n*3	0.83	0.29	0.84
20:5*n*3	6.96	5.32	6.19
22:1*n*11	5.11	9.58	5.63
22:1*n*9	0.79	0.65	
21:5*n*3	4.59	1.56	
22:4*n*6	1.04	0.57	
22:5*n*3	1.28	0.62	1.29
22:6*n*3	8.07	5.15	6.79
Misc.	4.34	3.88	8.75
Total *n*3	24.04	14.96	18.28
Total *n*6	4.23	5.47	6.57
*n*3:*n*6	5.68	2.73	2.78
Dry matter, %	90.3	91.5	90.3
Lipid, % of dry matter	15.9	17.8	15.8

ernment facilities did not permit equal numbers of fish to be sampled from each group for data on egg diameter, fertility, and eyeing. The actual numbers of fish sampled for measurement of each parameter are indicated in Table 2. Mean egg diameter was determined by placing a minimum of 10 eggs from a fish in a measuring trough calibrated in millimetres. The overall length of the row of eggs was divided by the number of eggs in the sample to give the mean diameter of eggs in the sample.

Fertilization

Eggs from each of the cultured broodfish were fertilized with mixed milt from two males. In the case of the wild fish, eggs from several females were combined and fertilized with milt from several males. In the latter instance, it was not, therefore, possible to follow the incubation success of eggs from individual females.

Incubation

The eggs of each female were incubated in separate Heath trays. Small lots of eggs were often combined after eyeing and the removal of infertile eggs and dead embryos. Data on the incubation success of eggs from many individual fish were consequently not available beyond the eyed egg stage.

In order to assess fertility, at least 10 eggs from each fish were taken 12–24 h after fertilization and placed in Stockard's solution (Velsen 1980) for clearing. Egg cleavage, indicating fertilization, was readily apparent by gross observation or with the aid of a dissecting microscope. Fertility could not be monitored at the government hatcheries because the eggs from individual fish were not incubated separately.

Eyeing success was calculated as a percentage of eggs set. The eyeing performance of the cultured RC fish was poor. Dead embryos were removed and the survivors from individual fish were combined to keep the Health trays full. Individual eyeing data for the RC groups were therefore unavailable. Because the eggs from individual females were not incubated separately at the government hatcheries, the eyeing success for the wild eggs was estimated on the basis of all the chinook spawned during the year in the respective hatcheries.

Analytical procedures

Samples of the two formulated commercial diets were analyzed for total lipid concentration and fatty acid (total lipid) composition. The exact composition of the natural diet consumed by the wild fish cannot be known.

Dry matter concentration was determined on subsamples of eggs by drying to constant weight in a hot-air oven at 70°C.

Lipid was extracted from homogenized egg samples according to the procedure of Bligh and Dyer (1959) with modifications by Christie (1982, p. 22). The total amount of lipid extracted was determined gravimetrically following evaporation of the solvent from aliquots of each extract. Butylated hydroxytoluene was added (50–100 mg/L) to the solvents used in the extraction and separation procedures and in the preparation of fatty acid methyl esters. Solvents were evaporated under reduced pressure and the vacuum was released with nitrogen.

Polar lipids were determined on aliquots of chloroform extracts containing approximately 200–250 mg of lipid. The solvent was removed in a rotary evaporator. The residual oil was applied, under nitrogen, by syringe to two Sep-Pak silica cartridges (Waters Associates, Milford, MA) placed in series. The neutral fraction of lipid was then eluted with a 60-mL mixture of chloroform and hexane (85:15, v/v). After elution of the neutral lipid, polar lipids were eluted with 60 mL of methanol. Aliquots of each eluate were evaporated and the amounts of lipid present determined gravimetrically after evaporation of the solvent. Separation of the neutral and polar lipids was verified by thin-layer chromatography using Silica G-plates (A. G. Darmsted, West Germany) and a solvent system consisting of hexane – diethyl ether – formic acid (80:20:2) according to Christie (1982, p. 93).

Fatty acid analysis

Fatty acid methyl esters were prepared from the total lipid and the polar lipid fractions by the method of Christie (1982, p. 53) for base-catalyzed methyl esters.

Fatty acid methyl esters were separated using a Varian 3700 gas chromatograph equipped with a Supelcowax 10 column (Supelco Canada Ltd. Oakville, Ont.), 30 m × 0.32 mm inside diameter. The carrier gas was helium. It was purified through a heated furnace (Supelco Canada Ltd.) to remove all traces of oxygen and water. The linear velocity was 22 cm/s. The thermal gradient was 170°C for 10 min, increased 2°/min to 230°C, and held at 230°C for 20 min. The injection and detection temperatures were 220°C and the split ratio 50:1.

Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman 1986). Quantification was accomplished using a Varian CDS

TABLE 2. Characteristics of eggs from wild (w) and cultured (c) Big Qualicum (BQ) and Robertson Creek (RC) chinook broodstocks. Experiment 1.

Source of eggs	Diameter, mm ± SEM (*n*)	Dry matter, % ± SEM (*n*)	Total lipid,[a] % ± SEM (*n*)	Polar lipid, [a] % ± SEM (*n*)	Fertility, % ± SEM (*n*)	Eyed[b] % ± SEM (*n*)
BQw	7.08 ± 0.27 (5)	39.5 ± 0.8 (5)	30.9 ± 0.6 (5)	12.8 ± 0.2 (5)	—[c]	90.9[d]
BQc (diet 1)	7.74 ± 0.04 (9)	39.6 ± 0.2 (5)	29.8 ± 0.4 (5)	12.8 ± 0.3 (5)	83.6 ± 7.2 (8)	62.4 ± 9.9 (16)
BQc (diet 2)	7.90 ± 0.04 (17)	39.3 ± 0.5 (5)	31.4 ± 0.3 (5)	13.2 ± 0.3 (5)	97.8 ± 2.2 (7)	73.8 ± 4.5 (44)
RCw	7.74 ± 0.06 (12)	40.3 ± 0.3 (5)	29.8 ± 0.3 (5)	12.8 ± 0.2 (5)	—[c]	90.3[d]
RCc (diet 1)	7.66 ± 0.12 (15)	35.9 ± 1.7 (5)	32.3 ± 0.8 (5)	14.2 ± 0.6 (5)	40.1 ± 0.7 (10)	36.3 ± 14.4 (8)
RCc (diet 2)	8.27 ± 0.13 (3)	39.6 ± 1.0 (3)	31.5 ± 0.7 (3)	13.9 ± 0.3 (3)	68.9 ± 26.3 (10)	94.6 ± 0.1 (2)

[a]% of dry matter.
[b]Eyed as a percentage of fertile eggs.
[c]Fertility was not assessed in the government hatchery.
[d]The percentage of eyed eggs is the hatchery estimate for all eggs set during that season.

401 recording integrator. Fatty acids representing less than 1% of the total have not been discussed.

The fatty acid profiles of the neutral lipid fractions were estimated from the proportions of polar and neutral lipid constituting the total lipid and the fatty acid compositions of the total and polar lipid factions.

Statistics

An arcsine transformation was used to normalize percentages in the fatty acid data. Analysis for differences in fatty acid composition for each of the three diet groups in each stock was performed using the general linear model procedure (Proc GLM) for a one-way analysis of variance, recommended for unbalanced data sets (SAS Institute Inc. 1985). The significance of differences between means was determined by least square means (LSMEANS) with the PDIFF option (SAS Institute Inc. 1985). The significance level was set at 95% for all tests.

Experiment 2: Comparison of Lipid Composition and Fatty Acid Profile of Alevins from Wild and Cultured Chinook Brookstock

Sources of alevins

Alevins from wild and cultured brookstock of the Chemainus (C) and BQ stocks were sampled twice in the late stages of incubation. The cultured broodstock of both stocks were from Sea Spring Salmon Farm Ltd. They had been fed a commercially formulated broodstock diet (Diet 3, Table 1) until transfer to freshwater for maturation and were not fed subsequently. The average water temperature during incubation was 9.8°C.

The wild C eggs were also incubated at Sea Spring Salmon Farm Ltd., but the alevins from the wild BQ fish were sampled at the Big Qualicum hatchery where the average water temperature was 9.1°C.

Each sample of alevins, analyzed for dry matter and lipid content, was a composite of 50-g lots of alevins representing three individual broodfish and taken from Heath trays or troughs at similar stages of development as measured by accumulated temperature units (ATU) (see Table 6).

Analytical procedures

Lipid was extracted from each of the homogenized alevin samples according to the procedures described above for Experiment 1. Aliquots of the lipid extracts were separated into neutral and polar fractions. The total lipid and the respective fractions were analyzed for fatty acid composition as described in Experiment 1 for egg samples.

Statistics

The mean values for the concentrations of each fatty acid in the lipid fractions of the alevins from the wild and cultured broodfish of each stock were compared by the *t*-test for equal or unequal variances ($p < 0.05$) as appropriate (SAS Institute Inc. 1985).

Results

Experiment 1

The lipid concentrations and the fatty acid profiles of the lipid in the two formulated diets (Diets 1 and 2) are shown in Table 1. The lipid concentration (dry weight basis) of Diet 1 was 15.9% and of Diet 2 ws 17.8%. Diets 1 and 2 included 24.0 and 15.0% of *n*3 fatty acids and 4.2 and 5.5% of *n*6 fatty acids, resulting in *n*3:*n*6 ratios of 5.7 and 2.7, respectively. The predominant fatty acids were C18:1*n*9 and 16.0. Diet 1 contained 17.1 and 17.0%, respectively, of these fatty acids and Diet 2 contained 19.4 and 16.8%.

Data on egg diameter, fertility, and embryonic survival to eyeing are presented in Table 2. Diameters were measured for the eggs of each of these fish and any others in the same group that spawned on the same day. Fertility could not be determined for every fish in the cultured groups because eggs are combined from fish that produce only a few eggs at the Sea Spring hatchery. Eyeing success was calculated from hatchery records for all fish in a stock/diet group, except for those egg lots that had been combined. Eyeing success was thus overestimated by an unknown factor because fish with poorer performance were excluded.

Fertility could not be determined for the eggs from the wild fish because the government hatcheries do not keep the eggs of individual fish separate at spawning. The data regarding percentage survival to eyeing for eggs from wild stock are average values from the Big Qualicum and Robertson Creek hatchery records.

TABLE 3. Fatty acid composition of total lipids in eggs from wild (w) and cultured (c) Big Qualicum (BQ) and Robertson Creek (RC) chinook broodstocks. Experiment 1. Within BQ and RC stocks, respectively, mean fatty acid concentrations in each row with the same superscript are not significantly different ($p < 0.05$).

	BQw (n = 5)		BQc (diet 1, n = 5)		BQc (diet 2, n = 5)		RCw (n = 5)		RCc (diet 1, n = 5)		RCc (diet 2, n = 3)	
Fatty acid	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM
14:0	2.43	0.23	2.08	0.04	2.39	0.12	2.54	0.26	2.20	0.06	2.29	0.07
14:1*n*9	1.71	0.46	1.73	0.59	1.11	0.19	0.91	0.17	2.01	0.53	1.04	0.13
16:0	11.85	0.41	11.71	0.22	11.90	0.16	12.17	0.32	12.15	0.22	11.15	0.10
16:1*n*9	0.42	0.10	0.60	0.06	0.57	0.08	0.54	0.08	0.36	0.15	0.54	0.04
16:1*n*7	7.76^a	0.17	5.72^c	0.13	6.42^b	0.07	7.01^a	0.14	5.99^c	0.13	6.47^b	0.10
18:0	5.12^a	0.04	4.21^b	0.04	4.36^b	0.09	5.24^a	0.07	4.34^c	0.12	4.77^b	0.14
18:1*n*9	18.71^c	0.53	23.73^b	0.26	26.55^a	0.25	19.56^b	0.66	24.96^a	0.64	26.16^a	0.20
18:1*n*7	3.89^b	0.15	4.30^a	0.05	3.87^b	0.08	2.95^b	0.09	4.06^a	0.04	4.15^a	0.06
18:1*n*5	0.42	0.03	0.45	0.01	0.43	0.01	0.47	0.05	0.44	0.01	0.42	0.01
18:2*n*6	0.79^c	0.05	3.43^b	0.07	4.79^a	0.14	0.84^c	0.07	3.85^b	0.06	4.59^a	0.14
18:2*n*4	0.32	0.02	0.00		0.00		0.27	0.03	0.00		0.00	
18:3*n*6	0.00		0.25	0.07	0.44	0.05	0.00		0.30	0.03	0.29	0.02
18:3*n*4	0.47	0.02	0.11	0.05	0.17	0.04	0.29	0.04	0.17	0.02	0.11	0.05
18:3*n*3	0.72	0.05	0.54	0.02	0.63	0.03	0.71	0.09	0.60	0.01	0.63	0.03
18:4*n*3	0.85	0.05	0.68	0.03	0.71	0.04	1.08^a	0.13	0.63^b	0.06	0.56^b	0.02
20:0	0.50	0.05	0.23	0.00	0.21	0.03	0.45	0.06	0.17	0.05	0.25	0.01
20:1*n*11	0.40	0.19	0.72	0.03	0.73	0.03	0.41	0.19	0.66	0.04	0.54	0.04
20:1*n*9	0.86^b	0.05	1.94^a	0.06	2.10^a	0.11	0.87^c	0.06	1.70^b	0.09	2.13^a	0.11
20:2*n*6	0.00		0.36	0.01	0.39	0.04	0.00		0.32	0.01	0.46	0.03
20:3*n*6	0.00		0.70	0.03	0.91	0.05	0.03	0.03	0.78	0.05	0.98	0.03
20:4*n*6	1.15^b	0.02	1.60^a	0.04	1.68^a	0.07	1.02^b	0.03	1.62^a	0.03	1.73^a	0.03
20:4*n*3	1.34^a	0.04	0.69^b	0.04	0.56^b	0.04	1.71^a	0.21	0.66^b	0.04	0.62^b	0.04
20:5*n*3	13.70^a	0.36	8.88^b	0.16	7.45^c	0.14	15.06^a	0.46	8.36^b	0.24	7.88^b	0.07
22:1*n*11	0.13	0.08	0.39	0.02	0.45	0.05	0.11	0.08	0.37	0.01	0.27	0.10
21:5*n*3	0.75	0.36	0.31	0.04	0.24	0.00	0.37	0.05	0.33	0.03	0.21	0.03
22:4*n*6	0.40	0.12	0.26	0.02	0.23	0.03	0.30	0.02	0.10	0.05	0.07	0.05
22:5*n*3	5.49^a	0.23	3.47^b	0.08	2.74^c	0.15	4.81^a	0.20	3.10^b	0.07	3.28^b	0.19
22:6*n*3	17.09^a	0.74	17.73^a	0.39	14.87^b	0.45	17.81^a	0.51	16.70^{ab}	0.25	15.63^b	0.09
Misc.	1.42		1.59		1.58		0.96		1.61		1.49	
Total *n*3	39.95^d	0.87	32.30^b	0.59	27.22^c	0.35	41.56^a	0.59	30.38^b	0.47	28.82^b	0.18
Total *n*6	2.34^c	0.07	6.61^b	0.06	8.47^a	0.22	2.19^b	0.13	6.97^a	0.07	8.12^a	0.14
*n*3:*n*6	17.07		4.89		3.21		18.98		4.33		3.55	

The eggs from BQ fish fed the formulated diets were significantly larger than those from the wild BQ stock. The dietary effect was not, however, evident in the case of the RC stock (Table 2). The high moisture/low dry matter concentrations in the RC eggs from broodstock fed Diet 1 are notable. Coincidentally, these eggs were inferior in performance to all other groups.

Eggs from five fish from each stock and diet group were analyzed for lipid composition except for the RC fish fed Diet 2 from which only three fish were available. The fatty acid composition of the egg lipids are shown in Table 3 (total lipids), Table 4 (polar lipid fraction), and Table 5 (neutral lipids). C18:1*n*9 was the fatty acid present at highest concentration in the total lipids of all samples of eggs regardless of parentage or diet. C18:1*n*9 was present at significantly lower levels in the wild eggs than in the cultured eggs. C22:6*n*3 was consistently present as the fatty acid at the second highest concentration in the total lipids. The concentration of this fatty acid was significantly greater in the eggs from the wild fish and the cultured fish fed Diet 1 than in the eggs from the cultured fish fed Diet 2.

Fatty acids of the *n*3 series represented 39–42% of the fatty acids in the total lipid in eggs from the wild fish in contrast with 27–32% of total fatty acids in the eggs from the broodstock fed the formulated diets. Eggs from wild fish contained significantly higher concentrations of C20:5*n*3 than did eggs from the cultured fish; the difference between the wild and cultured fish in total *n*3 fatty acid concentration was primarily due to the difference in the concentration of this fatty acid.

The polar fractions of egg lipids (Table 4) contained greater proportions of *n*3 fatty acids, and of C16:0 and 18:0, but lower proportions of C18:1*n*9 than were present in the neutral lipid. Again, the *n*3 fatty acids were found at greater concentrations in the wild eggs (45–47%) than in the cultured eggs (38–41%). C20:5*n*3 and C22:5*n*3 were the major contributors to the difference in the total concentration of *n*3 fatty acids in the polar lipids of wild and cultured eggs. Also notable in the polar fatty acid profile was the presence of C22:6*n*3 at high concentrations that were not significantly affected by diet.

Significantly lower concentrations of *n*6 fatty acids were present in the wild eggs than in the cultured eggs. This fact, together with the difference in *n*3 fatty acid concentrations, resulted in *n*3:*n*6 ratios that were 4.4–8.0 times higher in the neutral lipids of the wild eggs than in the cultured eggs and 2.6–3.5 times higher in the polar lipids of the wild eggs than in the cultured eggs.

TABLE 4. Fatty acid composition of polar lipids in the eggs from wild (w) and cultured (c) Big Qualicum (BQ) and Robertson Creek (RC) chinook broodstocks. Experiment 1. Within BQ and RC stocks, respectively, mean fatty acid concentrations in each row with the same superscript are not significantly different ($p < 0.05$).

	BQw (n = 5)		BQc (diet 1, n = 5)		BQc (diet 2, n = 5)		RCw (n = 5)		RCc (diet 1, n = 5)		RCc (diet 2, n = 3)	
Fatty acid	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM
14:0	1.32[a]	0.10	1.09[b]	0.04	0.92[b]	0.03	1.33[a]	0.09	0.99[b]	0.06	1.09[b]	0.06
14:1*n*9	2.44	0.69	5.64	0.79	3.40	0.42	2.23	0.50	3.83	1.66	2.30	0.35
16:0	16.41	0.38	15.22	0.37	15.55	0.27	16.28	0.64	15.73	0.25	15.37	0.49
16:1*n*9	0.39	0.06	0.45	0.06	0.35	0.09	0.21	0.07	0.32	0.12	0.00	
16:1*n*7	2.87[a]	0.19	2.05[b]	0.15	2.08[b]	0.10	2.43[a]	0.12	1.94[b]	0.08	2.18[ab]	0.12
18:0	10.07[a]	0.31	7.46[c]	0.25	8.48[b]	0.29	9.99[a]	0.13	8.08[b]	0.27	9.39[a]	0.27
18:1*n*9	9.93[c]	0.38	12.27[b]	0.39	13.73[a]	0.23	10.25[c]	0.32	12.26[b]	0.22	13.48[a]	0.48
18:1*n*7	3.72	0.19	4.04	0.13	3.76	0.08	2.77[b]	0.12	3.76[a]	0.07	3.73[a]	0.28
18:1*n*5	0.49	0.05	0.51	0.02	0.48	0.03	0.59	0.07	0.47	0.01	0.50	0.04
18:2*n*6	0.28[c]	0.04	1.39[b]	0.07	2.03[a]	0.04	0.34[c]	0.02	1.57[b]	0.05	1.83[a]	0.02
18:3*n*3	0.25	0.07	0.05	0.05	0.18	0.05	0.17	0.06	0.02	0.02	0.07	0.07
18:4*n*3	0.19	0.10	0.09	0.04	0.14	0.06	0.31	0.05	0.09	0.05	0.00	0.00
20:0	0.20	0.05	0.00		0.00		0.12	0.06	0.00		0.00	
20:1*n*11	0.12	0.09	0.31	0.02	0.35	0.02	0.17	0.11	0.23	0.06	0.00	
20:1*n*9	0.96[b]	0.15	2.31[a]	0.10	2.46[a]	0.14	1.06[c]	0.12	1.96[b]	0.12	2.83[a]	0.21
20:1*n*7	0.00		0.20	0.05	0.14	0.06	0.00		0.04	0.04	0.08	0.08
20:2*n*6	0.00	0.00	0.42	0.02	0.45	0.03	0.00		0.40	0.03	0.59	0.04
20:3*n*6	0.00[c]	0.00	0.77[b]	0.03	1.12[a]	0.03	0.00[c]		0.90[b]	0.05	1.10[a]	0.07
20:4*n*6	1.68[c]	0.04	2.43[b]	0.07	2.88[a]	0.12	1.55[b]	0.04	2.68[a]	0.05	2.84[a]	0.02
20:4*n*3	0.66[a]	0.09	0.29[ab]	0.04	0.25[b]	0.06	0.92[a]	0.11	0.30[b]	0.08	0.27[b]	0.14
20:5*n*3	14.97[a]	0.45	10.69[b]	0.25	10.59[b]	0.16	15.59[a]	0.76	11.05[b]	0.48	10.39[b]	0.03
22:1*n*11	0.25	0.25	0.40	0.37	0.02	0.03	0.00		0.00		0.00	
21:5*n*3	0.14	0.07	0.35	0.14	0.00		0.23	0.03	0.85	0.20	0.00	
22:4*n*6	0.45	0.09	0.47	0.01	0.56	0.02	0.64	0.05	0.55	0.09	0.41	0.09
22:5*n*3	5.22[a]	0.37	3.48[b]	0.04	3.19[b]	0.19	4.41[a]	0.38	3.35[b]	0.13	3.33[b]	0.36
22:6*n*3	24.14	0.96	24.69	0.33	23.59	0.73	25.16	0.42	25.56	0.25	23.93	0.41
Misc.	0.98		1.04		1.79		0.93		0.56		0.87	
Total *n*3	45.57[a]	0.86	39.66[b]	0.40	37.93[b]	0.59	46.81[a]	0.71	41.22[b]	0.87	37.99[c]	0.35
Total *n*6	2.41[c]	0.15	5.49[b]	0.08	7.04[a]	0.10	2.54[c]	0.11	6.10[b]	0.12	6.76[a]	0.21
*n*3:*n*6	18.91		7.22		5.39		18.43		6.76		5.62	

Experiment 2

The fatty acid composition of the diet fed to the cultured fish in this experiment is given in Table 1 (Diet 3). The dry matter and the total, neutral, and polar lipid concentrations in the different samples of alevins (including residual egg material) are given in Table 6. The dry matter and lipid concentrations in the alevins decreased with increasing ATUs as lipid was utilized to meet energy requirements during the period before the alevins began feeding.

The fatty acid concentrations in the alevins varied little within the cultured and wild groups regardless of stock or stage of development. Data from the samples were therefore pooled. The mean values for fatty acid concentrations of the total, neutral, and polar lipids in the wild and cultured alevins are shown in Table 7. There were significant differences between the wild and cultured alevins. As was evident in the case of egg lipids in Experiment 1, the differences reflected the fatty acid profiles of the respective broodstock diets. Likewise, the effects of dietary composition were similar to those on egg lipids in that the fatty acid composition of the neutral lipids was more affected by dietary fatty acid composition than was the composition of the polar lipids. The concentrations of *n*3 fatty acids in the neutral lipids of the alevins of wild and cultured origin were 35.4 and 23.2%, respectively. The comparable values were 43.0 and 38.2% in the polar lipids. As shown in Table 7, the differences in fatty acid concentrations between the wild and cultured alevins were generally highly significant for all three lipid fractions. A notable exception was the concentration of C22:6*n*3 for which there was no significant difference between the wild and cultured alevins in either the neutral or the polar lipid. The major fatty acid in the neutral lipid was 18:1 regardless of whether the alevins were the progeny of wild or cultured stocks. In the polar lipids, on the other hand, the predominant fatty acid was C22:6*n*3. Although C18:1 was not the dominant fatty acid in the polar lipid fraction, the concentrations present in the polar lipids were significantly greater in the progeny of the cultured fish.

The ratios of *n*3:*n*6 fatty acids were consistently higher in the wild stocks than in the cultured stocks. The respective ratios were 14.7 versus 3.5, 16.1 versus 2.9, and 17.9 versus 6.7 for the total, neutral, and polar lipid fractions. The principal fatty acids responsible for differences in these ratios were C20:5*n*3 and C18:2*n*6.

Discussion

The precise lipid composition of the diet consumed by wild fish cannot be known. Differences in size (Table 2) between BQ eggs of wild and cultured origin are not necessarily ascribable entirely, or even in part, to the differences between the natural and the formulated diets consumed by broodstock. Propagation of salmon stocks through hatchery incubation of

TABLE 5. Fatty acid composition[a] of neutral lipids in eggs from wild (w) and cultured (c) Big Qualicum (BQ) and Robertson Creek (RC) chinook broodstocks. Experiment 1.

Fatty acid	BQw, %	BQc (diet 1), %	BQc (diet 2), %	RCw, %	RCc (diet 1), %	RCc (diet 2), %
14:0	3.21	2.82	3.46	3.45	3.15	3.24
14:1*n*9	1.19	—	—	—	0.58	0.04
16:0	8.63	9.07	9.25	9.08	9.34	7.82
16:1*n*9	0.44	0.71	0.73	0.79	0.39	0.97
16:1*n*7	11.22	8.48	9.57	10.46	9.17	9.86
18:0	1.62	1.76	1.37	1.66	1.41	1.12
18:1*n*9	24.92	32.36	35.85	26.57	34.92	36.17
18:1*n*7	4.01	4.50	3.95	3.09	4.30	4.48
18:1*n*5	0.37	0.40	0.39	0.38	0.42	0.36
18:2*n*6	1.15	4.97	6.79	1.22	5.64	6.77
18:2*n*4	0.55	—	—	0.47	—	—
18:3*n*6	—	0.44	0.76	—	0.54	0.54
18:3*n*4	0.80	0.19	0.29	0.51	0.30	0.20
18:3*n*3	1.05	0.91	0.96	1.12	1.06	1.07
18:4*n*3	1.32	1.12	1.12	1.66	1.05	1.00
20:0	0.71	0.40	0.36	0.70	0.30	0.45
20:1*n*11	0.60	1.03	1.01	0.59	1.00	0.97
20:1*n*9	0.79	1.66	1.84	0.73	1.50	1.58
20:2*n*6	—	0.31	0.35	—	0.26	0.36
20:3*n*6	—	0.65	0.76	0.05	0.69	0.89
20:4*n*6	0.78	0.98	0.81	0.62	0.79	0.85
20:4*n*3	1.82	0.99	0.78	2.30	0.94	0.90
20:5*n*3	12.80	7.52	5.17	14.66	6.25	5.90
22:1*n*11	0.05	0.38	0.76	0.19	0.66	0.48
21:5*n*3	1.18	0.28	0.41	0.48	—	0.38
22:4*n*6	0.36	0.10	—	0.04	—	—
22:5*n*3	5.68	3.46	2.41	5.11	2.90	3.24
22:6*n*3	12.10	12.49	8.55	12.28	9.75	9.07
Total *n*3	35.98	26.76	19.45	37.60	21.88	21.58
Total *n*6	2.29	7.45	9.51	1.89	7.38	8.64
*n*3:*n*6	15.71	3.59	2.05	19.89	2.96	2.50

[a]Calculated from the fatty acid analysis of the total and polar egg lipids (Tables 2–4).

TABLE 6. Composition of alevins (including residual egg material) from wild (w) and cultured (c) Big Qualicum (BQ) and Chemainus (C) chinook broodstocks at different accumulated temperature units (ATU). Experiment 2.

	BQw, %		Cw, %		BQc, %		Cc, %
	787 ATU	798 ATU	869 ATU	878 ATU	790 ATU	845 ATU	845 ATU
Dry matter	24.6	23.7	22.1	18.3	24.7	19.4	21.4
Total lipid, % dw	34.7	31.8	30.0	30.7	31.2	25.9	31.8
Neutral lipid, % of total lipid	22.5	21.1	20.0	19.9	19.6	16.3	21.5
Polar lipid, % of total lipid	12.2	10.7	10.0	10.8	11.6	9.6	10.3

eggs has been demonstrated to result in larger egg size. Larger egg size may be characteristic of cultivated stocks because of avoidance of the selection pressures against large size that exist under the physical stresses of natural incubation (Van den Berghe and Gross 1989). Alternatively, large eggs result in large smolts that are better able to withstand environmental pressures when released and are more likely to survive to reproduce (Bilton and Smith 1973). On the other hand, a difference in egg size between wild and cultured broodfish might be the result of the longer prespawning period experienced by wild fish during which time the fish are not eating.

The logistics of conducting Experiment 1 within the normal operations of commercial and government hatcheries limited the data that could be obtained. There were, for example, differences in the numbers of fish from which data on fertility and survival to eyeing were collected (Table 2). It is, however, evident that survival to eyeing in the eggs from the cultured fish was generally lower than the overall 90% survival to eyeing indicated by hatchery data for wild eggs. There were also differences (not shown) in ovarian maturity and egg retention in the spawners, with superior performance in the wild fish. The variations in the fertility and early mortality among the egg samples from the cultured broodstock, despite the similarities in the fatty acid composition of the eggs, indicate that a deficiency of essential fatty acid was not involved in the generally poorer incubation results. Eggs hatched the following year from

TABLE 7. Fatty acid profiles of total, neutral, and polar lipids in wild and cultured chinook alevins (including residual egg material). Experiment 2. Fatty acid concentrations between wild and cultured fish significantly different at $*p < 0.05$ and $**p < 0.01$.

	Total lipid				Neutral lipid				Polar lipid			
	Wild (n = 3)		Cultured (n = 4)		Wild (n = 3)		Cultured (n = 4)		Wild (n = 3)		Cultured (n = 4)	
Fatty acid	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM	%	SEM
14:0	3.14	0.19	2.04**	0.02	3.66	0.17	2.44**	0.17	1.44	0.12	0.97**	0.01
16:0	11.97	0.16	10.74**	0.19	9.62	0.13	8.85**	0.13	18.63	0.54	16.79	0.84
16:1	9.05	0.06	8.02**	0.06	10.69	0.26	9.27**	0.26	3.76	0.09	3.52	0.17
18:0	3.79	0.39	3.94	0.06	2.50	0.11	2.42	0.11	7.82	0.50	7.74	0.46
18:1	23.69	0.21	31.28**	0.33	26.56	0.31	35.30**	0.31	15.99	0.33	19.62**	0.16
18:2*n*6	0.93	0.15	5.40**	0.09	1.15	0.02	6.48**	0.02	0.39	0.04	2.56**	0.11
20:1	1.69	0.14	2.14*	0.05	1.75	0.15	2.17*	0.15	1.39	0.33	1.70	0.05
20:4*n*6	1.44	0.08	2.14**	0.14	1.04	0.04	1.52*	0.04	2.00	0.05	3.17**	0.06
20:4*n*3	1.59	0.02	0.58**	0.02	1.94	0.02	0.68**	0.02	0.71	0.02	0.29**	0.01
20:5*n*3	12.59	0.61	6.80**	0.07	13.32	0.50	6.50**	0.50	11.58	0.86	8.56*	0.32
22:5*n*3	4.94	0.30	3.28**	0.06	5.34	0.20	3.26**	0.20	3.95	0.18	3.20**	0.08
22:6*n*3	16.51	0.35	15.64*	0.35	14.77	0.73	12.77	0.73	26.71	0.42	26.20	0.87
Misc.	8.72		7.99		7.66		8.35		5.63		5.67	
Total *n*3	35.3		26.3**		35.4		23.2**		43.0		38.2**	
Total *n*6	2.4		7.5**		2.2		8.0**		2.4		5.7**	
*n*3:*n*6	14.7		3.5		16.1		2.9		17.9		6.7	

cultured chinook broodstock fed Diet 1 showed eyeing in 90% of the eggs set (unpublished data). This is further evidence that the fatty acid composition of the commercial diets fed in Experiment 1 was not responsible for the poor incubation results in that experiment.

The data in Table 3 demonstrate that differences in the fatty acid profiles between the eggs of the cultured fish fed the respective formulated diets reflect the differences in the fatty acid composition of Diets 1 and 2. Differences in the fatty acid profiles of egg lipids between the two stocks fed the formulated diets were small. Differences in concentrations of individual fatty acids in egg lipid between the wild BQ and RC stocks may be due to genetic effects or to dietary or other environmental factors.

The concentrations of *n*3 fatty acids in egg polar lipids were less affected by the fatty acid composition of the broodstock diets than were those in the neutral egg lipid. This observation is contrary to the statement by Watanabe (1982) that dietary lipids have a greater effect on the fatty acid composition of phospholipids than on the fatty acid composition of neutral lipids. It is noteworthy that there were no significant differences among the concentrations of C22:6*n*3 in the polar lipids among the diet groups, including the eggs from wild fish of either stock.

In avian reproduction, formulation of lipoproteins in the liver for transport to the developing ova is dependent upon linoleic or longer chain *n*6 fatty acids (March and MacMillan 1990), and maximum egg size is not achieved in the absence of sufficient *n*6 fatty acids to enable the maximum rate of synthesis of lipoproteins. The essentiality of *n*3 fatty acids for salmonid reproduction has been well documented (see Greene and Selivonchick 1987). It is likely that, similar to the need of the chicken for C18:3 in the synthesis of vitellogenin, chinook salmon require C22:6*n*3 (or a dietary *n*3 precursor) for synthesis of vitellogenin. It appears that the concentrations of *n*3 fatty acids in the formulated diets used in this study were adequate to meet the requirements for egg formation and that where poor reproductive performance was observed, it was not, as has already been stated, related to a dietary insufficiency of essential fatty acid. In any case, eggs from cultured fish tended to be larger than eggs from wild fish despite the higher concentrations of *n*3 fatty acids in the latter. Further to this point, Leger et al. (1981) reported that, when rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) were fed a diet deficient in *n*3 fatty acids for 3 mo prior to spawning, there was an increase in egg lipid content, primarily as a result of an increase in the oil globule component of the egg. The oil globule consists almost entirely of triacylglcyerols. In Experiment 1, because the proportion of neutral lipid in the eggs (Table 2) was similar between diet groups, it may be concluded on the basis of the data of Leger et al. (1981) that the formulated diets provided adequate amounts of *n*3 fatty acids to meet requirements for normal egg formation. Essential fatty acids are required specifically during reproduction, first in the synthesis of polar lipids required for vitellogenin production by the broodfish, i.e. for egg formation, and then in the synthesis of the polar lipid component of membranes during embryonic development. Sometimes referred to as a "conservation" process for essential fatty acids, it would probably be more accurately considered as priority usage of these fatty acids at a specific time, rather than a saving or holding process as is suggested by the term conservation.

The results of the experiments described here, together with the results of experiments conducted by other investigators, show that there are certain common characteristics of the lipids present in salmonid eggs and in the developing embryos through to swim-up. These characteristics are apparent despite differences in the fatty acid composition of dietary lipid and possible effects of salmonid species or stock. The polar lipid fractions of eggs and alevins contain larger proportions of *n*3 fatty acids than the neutral lipid fractions, regardless of the concentrations of the *n*3 fatty acids in the diet. Although eggs, and alevins hatching from them, may vary considerably in the concentrations of essential fatty acids present in their constituent polar lipids, C22:6*n*3 is consistently the predominant fatty acid.

An unresolved question is the minimum amount of *n*3 fatty

acids that must be ingested for the formation of sufficient vitellogenin before these fatty acids become limiting, either for egg size or for the numbers of follicles in which development can be initiated and supported to mature egg size. As already mentioned, the numbers and size of eggs produced are reduced when broodstock are deficient in *n*3 fatty acids (Watanabe et al. 1984). The dietary fatty acid requirement during the period of vitellogenesis is confounded by the previous dietary history and the consequent pool of essential fatty acid in the body of the broodfish. While demonstrating that viable alevins are produced with a considerable range in the *n*3 concentration in their polar lipids, the present experiment provides no information regarding the subsequent growth rate and viability of alevins that are the progeny of broodstock consuming dietary lipids with different fatty acid profiles.

Acknowledgements

This work was supported by funding from the British Columbia Science Council, Sea Spring Salmon Farm Ltd., Chemainus, B.C., and the Natural Sciences and Engineering Research Council of Canada. The authors acknowledge the assistance of Ms. Y. Yole (Ageing Unit, Department of Fisheries and Oceans) for ageing the wild fish from scales; Mr. Don Lawseth, Manager of Robertson Creek Hatchery, and Mr. Grant Ladocoeur, Manager of Big Qualicum Hatchery, for assistance in the collection of wild broodstock, and Dr. David Groves and the staff at Sea Spring Salmon Farm Ltd. for cultured broodstock and assistance in sample collection.

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Methods for Nonlethal Gill Biopsy and Measurement of Na^+, K^+-ATPase Activity

Stephen D. McCormick

Anadromous Fish Research Center, U.S. Fish and Wildlife Service, P.O. Box 796, Turners Falls, MA 01376, USA

McCormick, S. D. 1993. Methods for nonlethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50: 656–658.

A gill biopsy, in which a small portion of gill tissue was removed from anesthetized fish, was shown to have no detrimental effect on subsequent survival, growth, and salinity tolerance of juvenile Atlantic salmon (*Salmo salar*). A method for measurement of Na^+, K^+-ATPase activity in this small amount of gill tissue is presented. These methods are useful for nonlethal monitoring of physiological smolt characteristics in salmonids and may have applications in the study of disease, toxicology, and physiological ecology of many fish species.

Une biopsie des branchies, qui consiste à prélever un petit fragment de tissu branchial chez des poissons anesthésiés, n'a pas eu d'effet nuisible sur la survie, la croissance et la tolérance à la salinité de juvéniles de saumons de l'Atlantique (*Salmo salar*). L'auteur décrit une méthode de mesure de l'activité de la Na^+, K^+-ATPase dans le petit morceau de tissu prélevé par biopsie. Cette méthode s'avère utile au contrôle non létal de caractéristiques physiologiques de smolts de salmonidés et pourrait sans doute avoir des applications dans l'étude de la pathologie, de la toxicologie et de l'écologie physiologique d'un grand nombre d'espèces de poissons.

Received June 25, 1992
Accepted September 3, 1992
(JB535)

Reçu le 25 juin 1992
Accepté le 3 septembre 1992

Na^+, K^+-ATPase is an ion-translocating enzyme present in all animal cells. It is present in extremely high concentration in salt-transporting tissues such as the vertebrate kidney, salt glands of birds, rectal glands of sharks, and chloride cells of teleosts (Karnaky 1986). In chloride cells, Na^+, K^+-ATPase creates ionic and electrical gradients that are used for salt secretion in seawater, and possibly for ion uptake in fresh water (Silva et al. 1977; Zadunaisky 1984; Pequeux et al. 1988; Avella and Bornancin 1990).

In addition to its fundamental importance to ion transport in fish, gill Na^+, K^+-ATPase activity is often used as an indicator of the parr–smolt transformation of juvenile salmonids in hatcheries and laboratories. Zaugg (1982) presented a simple and widely used method for measurement of gill Na^+, K^+-ATPase activity; however, this and other methods require relatively large amounts of gill tissue, which requires killing juvenile fish. This paper validates a method of gill biopsy and measurement of Na^+, K^+-ATPase that should be useful for nonlethal sampling of young salmonids and other fishes. In addition to monitoring physiological changes in anadromous salmonids, gill tissue is important in monitoring fish health and response to natural and anthropomorphic environmental change. Gill biopsies may also be useful for nonlethal sampling of fishes in these areas of research.

Materials and Methods

Fish

Juvenile Atlantic salmon (*Salmo salar*) of sea-run (undomesticated) parentage were obtained from the White River National Fish Hatchery (Bethel, VT, USA) and reared at the Anadromous Fish Research Center (Turners Falls, MA, USA) for several months prior to experiments. Fish were maintained in 1100-L tanks continuously supplied with fresh water (10°C) and aeration. Lighting was provided by overhead fluorescent lights and daylength was altered twice weekly to provide a simulated natural daylength. Seawater exposure was conducted in aerated 360-L tanks with continuous filtration through paper and charcoal.

Gill Biopsy

Gill filaments are removed while the fish are under anesthesia. A cartilaginous septum (present in some species, such as salmonids, but not others) holds filaments together for one half their length. Reflect back the operculum with rounded forceps. For 20–80 g fish, cut 4–6 filaments just above the septum with fine-point scissors (more or less tissue for smaller or bigger fish, respectively). Do not cut below the septum as this will cause substantial bleeding. As the tissue is cut, rotate scissors slightly so that filaments stay on the scissors. Be careful not to crush the sampled or remaining filaments. Immerse cut filaments in 100 µL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) in a 0.5-mL microcentrifuge tube and freeze within 0.5 h. Gill tissue can be stored at −20°C for up to 6 wk and at −80°C for 3 mo.

Validation of Gill Biopsy

Juvenile Atlantic salmon weighing 44–77 g were anaesthetized and length and weight recorded. Twenty fish were marked with a visual implant tag (Northwest Marine Technology Inc., Shaw Island, WA, USA) posterior to the eye, and 10 of these received a gill biopsy. A group of untagged fish was included to ensure that the visual implant tag did not affect growth. Water temperatures were maintained at 12.5 ± 1°C throughout the experiment. After 26 d, fish were anaesthetized and length and

TABLE 1. Specific growth rate (weight), condition factor, gill Na^+, K^+-ATPase activity, and plasma sodium after seawater challenge in control or gill biopsy groups of juvenile Atlantic salmon. Values are mean ± SE (n = 10 per group). There was no significant difference between the groups in any of the measured parameters ($P > 0.2$, Student's t-test). This experiment was conducted in autumn when gill Na^+, K^+-ATPase activity is at its lowest level.

	Control	Gill biopsy
Growth rate (%·d^{-1})	0.57 ± 0.09	0.56 ± 0.09
Condition factor 100·(weight/length^3)	1.16 ± 0.02	1.15 ± 0.01
Gill Na^+, K^+-ATPase activity (μmol ADP·mg protein^{-1}·h^{-1})	0.72 ± 0.07	0.89 ± 0.10
Plasma sodium (mM) (after seawater challenge)	180 ± 2	176 ± 2

weight were recorded. Gill tissue was removed from all tagged fish for later measurement of gill Na^+, K^+-ATPase activity. Ten fish receiving their first gill biopsy and 10 fish without gill biopsy were kept in fresh water for 24 h and were then transferred to 25 ppt seawater (Clarke et al. 1985). After 24 h, fish were anaesthetized and blood removed from the caudal vessels into heparin-treated syringes. Blood was centrifuged at 5000g for 5 min and plasma removed and frozen at −80°C. Plasma sodium concentrations were measured by ion-selective electrodes (AVL Scientific Corp., Rosewell, GA, USA).

Measurement of Gill Na^+, K^+-ATPase Activity

Measurement of Na^+, K^+-ATPase activity is similar to that presented by Penefsky and Bruist (1984) and McCormick and Bern (1989), modified for 96-well microplates. The ouabain-sensitive hydrolysis of adenosine triphosphate (ATP) is enzymatically coupled to the oxidation of nicotinamide adenine dinucleotide (reduced form, NADH), which is directly measured in a microplate reader. An assay mixture (solution A) containing 4 U lactate dehydrogenase (LDH)·mL^{-1}, 5 U pyruvate kinase (PK)·mL^{-1}, 2.8 mM phosphoenolpyruvate (PEP), 0.7 mM ATP, 0.22 mM NADH, and 50 mM imidazole (pH 7.5) is made just prior to the assay and is stable for 2–3 d at 4°C (all biochemical reagents from Sigma, St. Louis, MO, USA). Assay solution B is as above but also contains 0.5 mM ouabain. A salt solution containing 189 mM NaCl, 10.5 mM $MgCl_2$, 42 mM KCl, and 50 mM imidazole (pH 7.5) is prepared in advance and is stable for several weeks at 4°C. Mix the assay solutions (A and B) and salt solutions separately in a 3:1 ratio, and keep on ice. A few minutes before use, place the amount of assay mixture to be used in a 25°C water bath. Before each day's use, run a standard curve from 0 to 20 nmol ADP·well.$^{-1}$ A decrease in NADH will occur immediately and should become stable within 2–3 min. The slope of the standard curve should be −0.019 to −0.020 OD unit·nmol ADP^{-1}·$well^{-1}$.

Thaw samples immediately prior to assay and keep on ice throughout; activity will begin to decrease immediately after homogenization, so activity measurements should begin within 0.5 h of tissue homogenization. Add 25 μL of SEID (0.5 g of sodium deoxycholate in 100 mL of SEI) to the microcentrifuge tube and homogenize filaments in the tube using a motorized pestle (Kontes, Vineland, NJ, USA). Grind tissue for 10–15 s, visually ensuring that all tissue (except cartilage) has been fully homogenized. Centrifuge at 5000g for 30 s to remove insoluble material.

With the microplate on an ice pack or other cold, dry surface, add 10 μL of homogenate to each of four wells for every sample. The remaining homogenate is removed and saved for later analysis of protein content (Smith et al. 1985). Add 200 μL of the A-plus-salt mixture to two wells per sample and 200 μL of the B-plus-salt mixture to the other two wells per sample. The plate is then placed in a temperature-controlled plate reader (we use Thermomax, Molecular Devices Corp., Menlo Park, CA, USA) and the linear rate of NADH disappearance is measured at 340 nm for 10 min. The linear rate from 2 to 10 min in each pair of duplicate wells is determined, and Na^+, K^+-ATPase activity is calculated as the difference in ATP hydrolysis in the absence and presence of ouabain, expressed as micromoles of ADP per milligram of protein per hour.

Results and Discussion

There were no mortalities after performing gill biopsies on juvenile Atlantic salmon in this experiment, or in several other experiments in which more than 100 fish were sampled.

There was no significant difference in growth rate of control fish and those receiving a gill biopsy in the 3-wk period following the procedure (Table 1). A third group that did not receive visual implant tags had the same average growth rate (0.54%·d^{-1}) as the tagged fish. In gill tissue taken from the same individuals, there was no difference in gill Na^+, K^+-ATPase activity initially (first biopsy) or after 26 d (second biopsy; $P > 0.05$, Student's t-test). Similarly, there was no difference in gill Na^+, K^+-ATPase activity in the control and gill biopsy groups at the end of 26 d (Table 1).

One day after receiving a gill biopsy, 10 control and 10 treated fish were exposed to a seawater challenge. There was no significant difference in the ability of the two groups to regulate plasma sodium after 24 h in 25 ppt seawater (Table 1).

Bleeding does not normally occur when using this method. Some blood loss may occur if the gill arch is mistakenly cut below the septum, but even this will not result in the death of the fish. The small portion of the total gill filament tissue that is removed in this method (0.3–0.6% in 50-g fish) makes it unlikely that gill functions of the fish will be greatly affected. This is supported by our inability to detect differences in growth rate or salinity tolerance after gill biopsy. However, there is obviously local tissue damage and temporary exposure of the blood to the external medium. Although we have not experienced any disease in fish with gill biopsies, this may be an important consideration, particularly in a hatchery setting. The cut surface heals within several days, but we have observed no significant regeneration of the filaments over a period of several months. Zaugg and McLain (1971) reported that coho salmon (*Oncorhynchus kisutch*) with approximately 10% of the gill filamental tissue removed lived for the duration of a 2-h experiment. Remarkably, Nanba et al. (1971) found that common carp (*Cyprinus carpio*) were able to survive removal of 75% of gill filament tissue (six of eight gill arches).

The method for measurement of Na^+, K^+-ATPase presented above is highly sensitive and reproducible. The coefficient of variation of a single homogenate was 4.7% (n = 6); variation of several gill biopsies from the same gill arch was 11.0% (n = 6). Na^+, K^+-ATPase activity increased linearly with increasing amounts of homogenate from 5 to 25 μL (4–20 μg of protein).

Incubation temperature for measurement of gill Na^+, K^+-ATPase activity in the present study was 25°C. This tempera-

TABLE 2. Gill Na^+, K^+-ATPase activity ($\mu mol \cdot mg\ protein^{-1} \cdot h^{-1}$) in Atlantic salmon presmolt (mid-February) and smolt (mid-May) and ratio (smolt:presmolt) using the method outlined in this paper and that of Zaugg (1982; data from McCormick et al. 1987). Values are mean ± SE of 10–12 fish per group.

	Presmolt	Smolt	Ratio
Present method	3.7 ± 0.5	15.2 ± 1.6	4.1
Zaugg (1982) method	16 ± 1	60 ± 5	3.8

ture was chosen because it is within the thermal limits of salmonids and most other teleosts and can be readily compared with other enzymes and metabolic processes measured at this temperature. Because gill Na^+, K^+-ATPase activity of salmonids increases linearly with increasing temperature up to 37°C, the activity of this enzyme can be measured with validity at these higher temperatures (and thereby increases the sensitivity of the assay). This may not be true, however, of all enzymes in a species, or for Na^+, K^+-ATPase in other species, and should be validated for each species.

Although the use of a crude homogenate results in lower specific activity than use of partially purified membrane preparations (Zaugg 1982), the relative changes in gill Na^+, K^+-ATPase activity between presmolts and smolts are identical (Table 2). Of the approximately fourfold difference in specific activity of the two methods, approximately 50% is due to the removal of soluble proteins in the partial membrane purification, and 50% is due to the higher temperatures (McCormick and Bern 1989). The use of crude homogenates has the advantage of losing only a small amount of total Na^+, K^+-ATPase activity during sample preparation (less than 10% in the present method, up to 30% as reported by Zaugg 1982). This advantage permits more direct comparison of Na^+, K^+-ATPase activity in other tissues and with other enzyme activities. Crude homogenates have the disadvantage of including soluble proteins that lower specific activity and may interfere with activity measurements. The comparisons of Table 2 and the successful use of this method in other studies (McCormick and Bern 1989; unpublished data) indicate that these are not major concerns in the study of gill Na^+, K^+-ATPase activity in salmonids.

There are other sensitive measures of gill Na^+, K^+-ATPase activity in fishes (e.g. Mayer-Gostan and Lemaire 1991), and it should be possible to modify these for use with gill biopsies. By modifying the salt mixture or inhibitors, these methods can be used to measure other ion-transporting ATPases (unpublished data; Mayer-Gostan and Lemaire 1991). After becoming proficient, the time required for the outlined method of gill biopsy is the same or less than that necessary for removing large pieces of gill tissue from the gill arch. No expensive equipment is required, although a pair of fine scissors is recommended. Although investigation of the parr–smolt transformation has been emphasized, these methods may be useful in research on pollution, disease, development, and environmental change in fishes.

Acknowledgments

I thank Michael O'Dea for his excellent technical assistance and the personnel of the White River National Fish Hatchery and Region 5, U.S. Fish and Wildlife Service, for providing the fish used in this study. Drs. Terry Bradley, Björn Th. Björnsson, and Henry Booke and an anonymous reviewer made many helpful comments in review of the manuscript.

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Mitochondrial DNA Variation in Lake Sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and James Bay Drainage Basins in Quebec, Canada

S. Guénette, R. Fortin, and E. Rassart

Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succ. A, Montréal (Québec) H3C 3P8, Canada

Guénette, S., R. Fortin, and E. Rassart. 1993. Mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and James Bay drainage basins in Quebec, Canada. Can. J. Fish. Aquat. Sci. 50: 659–664.

Restriction fragment length pattern analysis of mitochondrial DNA (mtDNA) was used to assess genetic differentiation in sympatric lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River drainage basin (St. Lawrence and Ottawa rivers and Lac des Deux Montagnes) and in sturgeon from the Waswanipi River (James Bay drainage basin). Using 14 restriction enzymes on mtDNA from 82 specimens, the genetic divergence was relatively low (d = 0.219–0.744%), as only three genotypes were found. Genotype 1 is present at all sites whereas genotype 2 is present only in Lac des Deux Montagnes and the Ottawa and Waswanipi rivers. The St. Lawrence River sturgeon form a homogeneous genotypic group that was not found significantly different from sturgeon from Lac des Deux Montagnes. The genetic heterogeneity seemed higher in the James Bay drainage population than in the St. Lawrence drainage populations that probably have been more significantly influenced by overfishing and man-made habitat changes.

Nous avons utilisé la comparaison des fragments générés par des endonucléases de restriction pour étudier les variations de l'ADN mitochondrial des Esturgeons jaunes (*Acipenser fulvescens*) du bassin de drainage du fleuve Saint-Laurent (fleuve St-Laurent, rivière des Outaouais et lac des Deux Montagnes) et des esturgeons de la rivière Waswanipi (bassin de drainage de la Baie James). La différenciation génétique estimée par l'utilisation de 14 enzymes de restriction sur 82 spécimens est relativement faible (d = 0,219 à 0,744%) puisque seulement trois génotypes ont été identifiés. Le génotype 1 est présent dans tous les échantillons tandis que le génotype 2 n'est présent que chez les populations du lac des Deux Montagnes et des rivières des Outaouais et Waswanipi. Les esturgeons du Saint-Laurent sont homogènes au niveau génotypique et ils ne diffèrent pas significativement de ceux du lac des Deux Montagnes. L'hétérogénéité génétique semble plus élevée pour les populations du bassin du Saint-Laurent, qui ont probablement été plus influencées par les pêches excessives et par les modifications anthropiques de l'habitat.

Received March 4, 1992
Accepted August 26, 1992
(JB425)

Reçu le 4 mars 1992
Accepté le 26 août 1992

Like the other members of the family Acipenseridae, lake sturgeon (*Acipenser fulvescens*) are slow-growing, late-maturing, and long-lived fish. Published ages at first spawning are usually greater than 13 yr for males and greater than 19 yr for females (Houston 1987). After sexual maturity is reached, there may be an interval of 2 yr between successive spawnings in males and 4–6 yr, and even 8–9 yr, in females (Roussow 1957; Sunde 1961; Cuerrier 1966; Magnin 1966; Goyette et al. 1987). Magnin (1966) mentioned usual maximum ages of 55 yr for males and 80 yr for females. Although lake sturgeon are not endangered or even rare in Canada, the threat of serious declines leading to extinction may be imminent if the necessary stock assessments and habitat protection measures are not forthcoming (Houston 1987). Limiting factors include commercial exploitation, construction of dams and barriers on large rivers that prevent migration to suitable spawning habitats, pollution, habitat degradation, and related environmental stress resulting from industrial, urban, and rural development (Houston 1987).

In Quebec, the lake sturgeon is present in the St. Lawrence River drainage, including one of the most important tributaries, the Ottawa River and its enlargement Lac des Deux Montagnes. It is also present in various lakes and rivers in the James Bay drainage. In view of the rate of substitution in vertebrate mitochondrial DNA (mtDNA) (2% per million years, Brown et al. 1979) and given the fact that sturgeon from the James Bay and St. Lawrence drainages have been isolated for only approximately 8000 yr (Crossman and McAllister 1986) after their dispersion from the Mississippi glacial refuge, we might not expect a very significant population subdivision between and within the two drainages. However, the various limiting factors mentioned above, which may act as genetic bottlenecks, could have had strong impacts on mtDNA genotypes of southern populations. To obtain a broader view of the population genetics of lake sturgeon, we studied mtDNA variation in specimens from various sites in the St. Lawrence and from one site in the James Bay drainages in Quebec. Broadening our knowledge of the population genetics of lake sturgeon might become an important element in the conservation and management of this species in the near future.

We investigated possible substructuring of populations in the St. Lawrence drainage, including the Ottawa River. We first

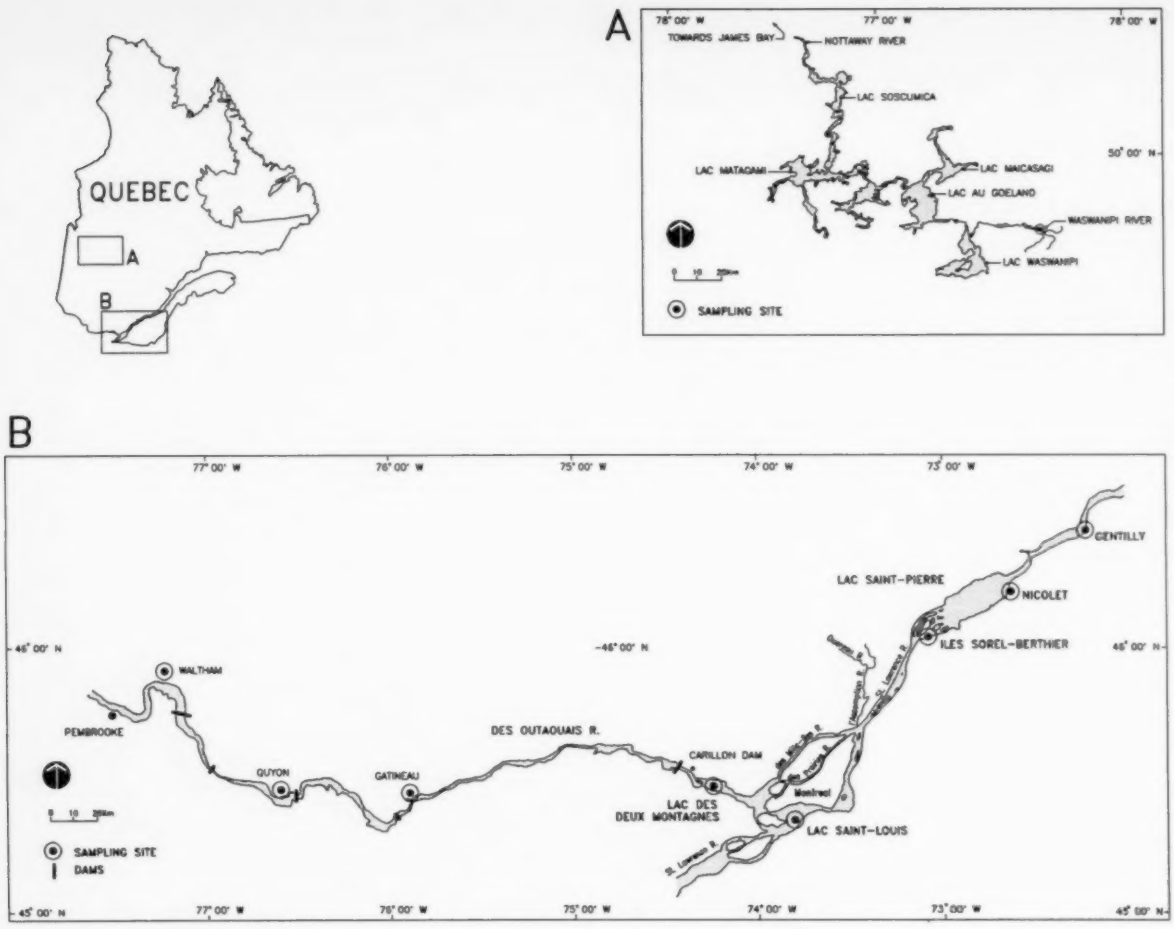

FIG. 1. Areas sampled in the St. Lawrence River, the Montreal Archipelago, the Ottawa River, and the Waswanipi River.

chose four sampling sites in the St. Lawrence River (Lac Saint-Louis, Îles Sorel-Berthier and Nicolet in Lac Saint-Pierre, and Gentilly; Fig. 1). Two previous studies suggested that these fish might be homogeneous. Guénette et al. (1992), using an extensive set of morphological characteristics, showed that lake sturgeon from these four locations in the St. Lawrence River form a relatively homogeneous phenotypic group. Moreover, Dumont et al. (1987) showed that although commercial-size sturgeon (mostly subadults) tagged in these various commercial fishing areas seem rather sedentary, adults tagged on spawning grounds in the Des Prairies and L'Assomption rivers (Fig. 1) were recaptured in the St. Lawrence River from Lac Saint-Louis to Lac Saint-Pierre. Thus, sturgeon from the St. Lawrence River seem to use common spawning grounds and possibly form a homogeneous genetic group.

Sturgeon from Lac des Deux Montagnes are sympatric with St. Lawrence fish because free movement is possible between the two water bodies. A number of interesting points emerge when one compares the mtDNA structure of Lac des Deux Montagnes sturgeon with that of St. Lawrence fish and fish from the Ottawa River, upstream of the Carillon dam, which was built in the early 1960s and prevents upstream movement of potential migrants, but not downstream movement. First, Lac des Deux Montagnes sturgeon differ morphologically from St. Lawrence fish (Guénette et al. 1992). They have a slower growth rate and a lower condition factor. They are also considered to be highly sedentary within Lac des Deux Montagnes. They do not use St. Lawrence River spawning grounds (Mongeau et al. 1982; Fortin et al. 1991). Thus, they could be genetically distinct from St. Lawrence fish. Second, during the winter of 1960, a major anoxia, probably caused by industrial pollution, killed most of the sturgeon population in Lac des Deux Montagnes (Mongeau et al. 1982). Since then, the population has been slowly recovering. However the origin of the present population in the lake is unknown because the genetic relationship between these sturgeon and those from downstream (St. Lawrence River) and upstream (Ottawa River, above Carillon dam) remains unstudied. These considerations lead us to choose three sampling sites on the Ottawa River, located in three different impoundments, for comparison with Lac des Deux Montagnes and the St. Lawrence River. Specimens from Waltham and Quyon were pooled in a single sample and compared with the sample from Gatineau.

Materials and Methods

Isolation of mtDNA

Our specimens were obtained from commercial fishermen during the summer of 1989, except those from Lac des Deux Montagnes, which were captured by our field team. The livers of 12 sturgeon from each site were used for mtDNA extraction. Livers were kept on ice and processed within 3–12 h. mtDNA was extracted following a protocol modified from Lansman et al. (1981). Briefly, 10 g of liver was homogenized and centrifuged two times each at 700 × *g* and 20 000 × *g* to isolate mitochondria that were later lysed with 1% sodium dodecylsulfate (SDS). The solution, mixed with 1.075 g $CsCl_2$/mL, was kept at room temperature in the dark for 12 h to 2 wk. Ethidium bromide was added to a final concentration of 0.45 mg/mL just before transferring the solution into ultracentrifuge tubes. Samples were centrifuged for 72 h at 160 000 × *g* (20°C). Ethidium bromide was extracted with isopropanol saturated with NaCl (5 M).

Restriction Enzyme Analysis

Restriction enzymes were purchased from Pharmacia PL (Montréal) and the digestions were done under the conditions recommended by the supplier. Fourteen different restriction enzymes were tested. Restriction fragments were separated by electrophoresis on horizontal 1.2% agarose gel. The DNA of bacteriophage lambda, digested by *Hind*III, was used as a molecular weight standard.

Southern Transfer, Hybridization, and Probe

The mtDNA fragments were transferred from the agarose gel to a nylon membrane (Hybond-N, Amersham), according to Maniatis et al. (1989). The DNA was covalently fixed to the membrane by a UV exposure of 3–4 min and the membrane was prehybridized for 8–16 h. The nylon membranes were hybridized in 50% formamide – 3X SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.05% SDS, 5X Denhardt solution, and the heat-denatured ^{32}P-labeled probe at 42°C for 10–16 h. After hybridization, the membranes were washed sequentially in 2X SSC–0.1% SDS three times for 5 min at 22°C and in 0.1 SSC – 0.1% SDS for 60 min at 65°C. The membranes were dried and exposed at −80°C to XAR-5 Kodak film (Eastman Kodak Co., Rochester, NY) using a Cronex Lightning-plus intensifying screen (DuPont Co., Wilmington, DE) for 4–72 h. The probe was made from purified mtDNA. Supercoiled mtDNA was cleaved by *Eco*RI to produce two fragments of 9 kbp that were further purified by eluting them from the agarose gel. Labeling of the probe was done on 20 ng of purified fragment by the random primer extension method (Feinberg and Vogelstein 1983) with oligohexamers (Pharmacia PL Biochemicals, Montréal, Québec). The specific activity varied between 1 and 2 × 10^9 cpm/μg DNA.

We tested 14 different restriction endonucleases on the mtDNA. Seven of them were rejected because they did not cleave lake sturgeon mtDNA (*Kpn*I and *Eco*RV on 18 specimens and *BamH*I, *Sma*I, *Cla*I, *Mlu*I, and *Xho*I on one specimen). The others cleaved at least three times and were kept for further analysis: three six-base sequences (*Bcl*I, *Sac*II, and *Hpa*I), one four-base sequence (*Taq*I), two multiple six-base sequences (*Hinc*II and *Ava*I), and one five-base multiple sequence (*Ava*II).

Statistical Analysis

The number of nucleotide substitutions per site (*d*) was estimated on the basis of the number of common restriction fragments, following Nei (1987, p. 106–107). The estimates were made separately for each group of endonucleases sharing the same number of nucleotides in the restriction sequence (*r*) which was calculated following Nei and Tajima (1981). The total genetic distance between two genotypes is the weighted mean for the number of fragments generated by each group of enzymes. The intrapopulation diversity index (*h*) was calculated for each geographic sector, following Nei and Tajima (1981), in order to compare lake sturgeon populations with other species of sturgeon. This index accounted for the number of genotypes and their frequency. Geographic heterogeneity was compared with the likelihood ratio test (adjusted *G*, Sokal and Rohlf 1981).

Results

Based on undigested mtDNA, lake sturgeon mtDNA is probably about 17.5 kbp long whereas the mean length obtained with digestion patterns would be 16 kpb (± 1.3 SE). However, migration patterns could be consistently determined. The seven enzymes detected 41–44 restriction sites representing about 1.3% of the genome (224 bp).

No case of heteroplasmy (multiple mtDNA length variant within an individual) was found. Length polymorphism (multiple mtDNA length variant between individuals) was detected with four enzymes (*Hinc*II, *Bcl*I, *Sac*II, and *Ava*II). The "short" type of mtDNA was consistently about 200 bp shorter than the "long" type for all enzymes (Table 1). In St. Lawrence River, Lac des Deux Montagnes, and Ottawa River sturgeon, the long type of mtDNA was twice as abundant as the short type whereas they were equally represented in Waswanipi River sturgeon (Table 2). Length polymorphism does not seem to be linked to the genotype, but sturgeon that show the long type and genotype 1 are more abundant in Waswanipi (n = 6/12) than in southern locations (n = 22/70) whereas all genotype 2 fish from Waswanipi show the short type.

Only three enzymes (*Hinc*II, *Ava*II, and *Bcl*I) detected restriction site polymorphism (Table 1). Differences between two migration patterns were caused by the loss or gain of a single restriction site. Two different patterns for a single enzyme were labeled A and B, respectively. All sturgeon from the St. Lawrence River showed genotype 1 except one fish from Lac Saint-Louis that had genotype 3. Consequently the heterogeneity index of the St. Lawrence River fish is the lowest (h = 0.04). Lac Saint-Louis and Lac des Deux Montagnes have similar indices (h = 0.014). The two sampling sites in the Ottawa River showed no differences in genotype frequency and thus will be considered as a single sample in the following discussion. Genotype frequency is not significantly different among Lac des Deux Montagnes, Ottawa River, and Waswanipi River sturgeon (adjusted G = 2.62, $p > 0.05$). The heterogeneity index is similar for Lac des Deux Montagnes and Ottawa River sturgeon (0.18 and 0.22, respectively) but twofold higher for Waswanipi River fish (h = 0.48). The overall index is 0.17 for the 82 sturgeon studied. The distance (d) between genotypes varied from 0.22 to 0.74%; the distance between genotype 1 and 2 is 0.52 and the maximal distance occurred between genotypes 2 and 3.

TABLE 1. Restriction enzyme patterns of mtDNA (kbp). The fragments showing length polymorphism are in parentheses. The "long" fragment is 200 bp longer than the indicated length.

*Hinc*II		*Ava*II		*Bcl*I					
A	B	A	B	A	B	*Sac*II	*Ava*I	*Taq*I	*Hpa*I
5.0	5.0	(3.5)	(3.5)	5.9	5.9	10	6.0[a]	5.3	11
(4.5)	—	—	2.9[b]	(5.2)	(5.2)	(4.8)	4.1	1.8[a]	3.4
—	(3.05)	2.2	—	2.3	2.3	1.7	1.3	1.6	3.1
2.9	2.9	1.8	1.8	—	2.15			0.97	
2.0	2.0	1.35	1.35	1.35	—			0.85	
—	1.45	1.28	1.28	0.8	0.8			0.7	
0.9	0.9	1.05	1.05	0.7	—			0.5	
0.5	0.5	0.7[a]	0.7						
		0.54[a]	0.54[a]						
		0.44	0.44						
		0.38	0.38						

[a]The band detected is a doublet.

[b]For pattern B, the 0.7-kbp band is less intense than for pattern A. Thus, the 2.9-kbp fragment is composed of the 2.2- and one 0.7-kbp fragment.

TABLE 2. Distribution of the short and long forms of mtDNA and of the three genotypes in the various areas studied. The letters of the composite profile refer to the enzymes in the following order: *Hinc*II, *Ava*II, *Bcl*I, *Sac*II, *Ava*I, *Taq*I, and *Hpa*I.

		Genotype				
		1 AAAAAAA		2 BBAAAAA		3 AABAAAA
Sector	*n*	Short	Long	Short	Long	Short
St. Lawrence River						
Lac Saint-Louis	11	7	3	0	0	1
Lac Saint-Pierre						
Sorel	9	5	4	0	0	0
Nicolet	11	9	2	0	0	0
Gentilly	11	7	4	0	0	0
Lac des Deux Montagnes	11	8	2	0	1	0
Ottawa River						
Waltham–Quyon	8	5	2	0	1	0
Gatineau	9	6	2	0	1	0
Waswanipi River	12	2	6	4	0	0
Total	82	49	25	4	3	1

Discussion

The mechanism causing length polymorphism in lake sturgeon is unknown, but we suggest that it could be due to the repetition of a fragment, as is the case in white sturgeon (*Acipenser transmontanus*). Buroker et al. (1990) attributed mtDNA length polymorphism in that species to either simple or multiple repetition of an 82-bp fragment.

The comparison of restriction fragments is used only when the expected divergence is low ($d < 0.1$). This technique implies more risk for error in the identification of fragments and may cause a larger variance than would the mapping of restriction sites (Nei 1987). However, our results are similar to those obtained by Brown (1991, $d = 0.3142\%$) who compared four lake sturgeon from lac Saint-Pierre and eight from the Nelson River (Hudson Bay drainage basin). The index of diversity found for lake sturgeon in the present study ($h = 0.174$) is smaller than the value mentioned by Brown et al. (1992) for sturgeon ($h = 0.65$). The low heterogeneity of lake sturgeon compares with that observed in pallid (*Scaphirhynchus albus*) and shovelnose sturgeon (*S. platorynchus*) ($h = 0.17$ and 0.01, respectively; Phelps and Allendorf 1983), two species that could not be differentiated on the basis of the comparison of 27 allozymes. Because the present study analyzes only 1.3% of the genome, it could be suggested that using more enzymes would reveal other differences. However, this might not be the case because the seven additional enzymes we tried did not cut the mtDNA and thus would not reveal any differences. Brown (1991) used 14 enzymes and found polymorphisms with only two of them, *Ava*II and *Hinc*II, which supports our conclusions. However, it could be possible, by using more direct techniques, to reveal further differences. For example, the sequencing of a portion of mtDNA (cytochrome *b*) lead to the differentiation of populations of Atlantic cod (*Gadus morhua*) from Newfound-

land and Norway (Carr and Marshall 1991) whereas other techniques had failed. The D-loop region has been found to be the mtDNA segment that is the most rapidly evolving and polymorphic region in humans (Viligant et al. 1991) and might also be used successfully in fish.

Sturgeon from different locations in the St. Lawrence River seem rather homogeneous in spite of the high number of individuals studied. The absence of genotype 2 in the St. Lawrence and its presence in Lac des Deux Montagnes support the hypothesis that sturgeon do not circulate between the two water bodies. After 1950, the drastic reduction of Lac des Deux Montagnes sturgeon acted as a population bottleneck; experimental fisheries in the 1960s showed the presence of two strong cohorts, 1955–56 and 1960–61, and no individuals born before 1951 (Mongeau et al. 1982). Again in 1978, these two cohorts dominated the catch. The authors concluded that the population depended on the recruitment in two particularly productive years. Our results suggest that recruitment came from the Ottawa River before the end of the construction of the Carillon dam between 1959 and 1964, although the possibility of immigration of sturgeon from the St. Lawrence River cannot be excluded.

Sturgeon from Lac des Deux Montagnes and the Ottawa and Waswanipi rivers are similar because of the presence of genotypes 1 and 2, which is in keeping with the presumed dispersion of lake sturgeon from the Mississippi glacial refugium. Lake Ojibway–Barlow, which contributed to the formation of the Hudson and James Bay basin, discharged into the Ottawa River until the connections were cut about 8000 yr ago (Crossman and McAllister 1986). During the same period, the Great Lakes were connected with the St. Lawrence River (Underhill 1986). Because all these lakes and rivers were once connected, the absence of genotype 2 in the St. Lawrence River is difficult to explain. However, Avise et al. (1984) showed with simulations that lineage extinction is a function of the interval since colonization, reproduction schedule, size of the founding population, and population size at carrying capacity. The first two factors are probably of the same scope for northern and southern populations whereas the size of the founding population is unknown. The variation in population size could be large enough to influence genotypic diversity in the Ottawa River and Lac des Deux Montagnes. Important modifications of the habitat and important commercial fishing pressure could also cause a decrease in genetic variability (Nelson and Soulé 1987). Brown et al. (1992) suggested that recent overexploitation and habitat destruction of white sturgeon acted as a severe genetic bottleneck which is responsible for the low genetic diversity observed in the Columbia River compared with the Fraser River. Thus, these factors could explain the lower genetic diversity of the populations in the Ottawa River, Lac des Deux Montagnes, and the St. Lawrence River compared with the Waswanipi River population. However, they do not account for the absence of genotype 2 in the St. Lawrence. If this absence was the consequence of extinctions due to the loss of spawning and feeding habitat in the St. Lawrence River, then the status of the Ottawa River sturgeon population would be expected to be the same, as dam construction and intensive commercial fishing seem to have considerably diminished that population as well (P. Dumont, Ministère du Loisir, de la Chasse et de la Pêche, Montréal, pers. comm.).

Lac des Deux Montagnes sturgeon were found to be morphologically different from the St. Lawrence River fish (Guénette et al. 1992). These morphological differences are thought to be the result of environmental conditions. However, the two populations are rather similar on the basis of our mtDNA analysis. This suggests either that phenotype and genotype are not closely linked and/or that our estimate of the genetic variation is not precise enough. Similar discrepancies between conclusions drawn from genetic and morphological information have been found in dwarf and normal forms of lake whitefish (*Coregonus clupeaformis*) which were considered similar on the basis of mtDNA analysis (Bernatchez and Dodson 1990). Shields et al. (1990) also concluded that dwarf and normal forms of cisco (*Coregonus artedi*) were genotypically similar for mitochondrial and chromosomal DNA. The homogeneity of genotypes throughout the St. Lawrence River and the low index of heterogeneity reinforce the importance of considering this sturgeon population as a whole for habitat protection purposes.

Acknowledgements

Financial support was provided by the Ministère du Loisir, de la Chasse et de la Pêche du Québec (MLCP), by the federal Department of Fisheries and Oceans, and by Université du Québec à Montréal. We would like to thank James R. Brown and Michael J. Smith from Simon Fraser University (Burnaby, B.C.) for sharing their experience in sturgeon mtDNA extraction. Special thanks to James R. Brown for comments on the manuscript. We also thank M. Pierre Dumont for helpful comments on an earlier draft. We gratefully acknowledge Richard Bergeron, Nicole Couture, and Xingbo Wang from Université du Québec à Montréal for their constant support throughout the laboratory work. Finally, we thank Paul Bentzen for his constructive comments in the early stages of the project.

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Dissolved Inorganic Nitrogen Composition, Transformation, Retention, and Transport in Naturally Phosphate-Rich and Phosphate-Poor Tropical Streams

Frank J. Triska

U.S. Geological Survey, Water Resources Division, 345 Middlefield Rd., MS 496, Menlo Park, CA 94025, USA

Catherine M. Pringle

Section of Ecology and Systematics and Center for the Environment, Cornell University, Ithaca, NY 14853, USA

and Gary W. Zellweger, John H. Duff, and Ronald J. Avanzino

U.S. Geological Survey, Water Resources Division, 345 Middlefield Rd., MS 496, Menlo Park, CA 94025, USA

Triska, F. J., C. M. Pringle, G. W. Zellweger, J. H. Duff, and R. J. Avanzino. 1993. Dissolved inorganic nitrogen composition, transformation, retention, and transport in naturally phosphate-rich and phosphate-poor tropical streams. Can. J. Fish. Aquat. Sci. 50: 665–675.

The composition, transformation, and transport of dissolved inorganic nitrogen (DIN) was compared in waters associated with two lowland streams in Costa Rica. The Salto River is enriched by geothermal-based soluble reactive phosphorus (SRP), which raises the concentration up to 200 μg/L whereas Pantano Creek, an unimpacted tributary, has an SRP concentration <10 μg/L. Ammonium concentration in springs adjacent to the Salto and Pantano was typically greater than channel water (13 of 22 locations) whereas nitrate concentration was less (20 of 22 locations). Ground waters were typically high in ammonium relative to nitrate whereas channel waters were high in nitrate relative to ammonium. Sediment slurry studies indicated nitrification potential in two sediment types, firm clay (3.34 μg $N \cdot cm^{-3} \cdot d^{-1}$) and uncompacted organic-rich sediment (1.76 μg $N \cdot cm^{-3} \cdot d^{-1}$). Ammonium and nitrate amendments to each stream separately resulted in nitrate concentrations in excess of that expected after correction for dilution using a conservative tracer. SRP concentration was not affected by DIN amendment to either stream. SRP concentration in the Pantano appeared to be regulated by abiotic sediment exchange reactions whereas DIN composition and concentration were regulated by a combination of biotic and abiotic processes.

On a comparé la composition, la transformation et le transport de l'azote inorganique dissous (AID) trouvé dans de l'eau associée à deux cours d'eau qui s'écoulent dans des basses terres du Costa Rica. Les eaux de la rivière Salto sont enrichies par du phosphore réactif soluble d'origine géothermale (PRS), qui porte la concentration à un maximum de 200 μg/L, alors que le ruisseau Pantano, un tributaire qui n'est pas soumis à cet effet, a une concentration de PRS inférieure à 10 μg/L. La concentration d'ammoniac dans des sources contiguës à la Salto et au Pantano est ordinairement supérieure à celle de l'eau du chenal (13 stations sur 22), alors que la concentration d'azote est inférieure (20 stations sur 22). L'eau souterraine est normalement riche en ammonium par rapport à la teneur en nitrate, alors que l'eau dans les chenaux est riche en nitrate comparativement à la teneur en ammonium. Des études faites sur une bouillie de sédiments ont montré qu'il existe un potentiel de nitrification de deux types de sédiments, une argile compacte (3,34 μg $N \cdot cm^{-3} \cdot d^{-1}$) et un sédiment non compact et riche en matières organiques (1,76 μg $N \cdot cm^{-3} \cdot d^{-1}$). Des amendments pratiqués avec l'ammonium et le nitrate dans chaque cours d'eau séparément ont conduit à l'obtention de nitrate en concentration supérieure aux résultats attendus, après correction pour la dilution au moyen d'un traceur persistant. Peu importe le cours d'eau, la concentration en PRS n'a pas varié par suite d'un traitement à l'AID. La concentration en PRS du Pantano paraissait être régulée par des réactions abiotiques d'échange avec les sédiments alors que la composition et la concentration en AID semblaient régulées par une combinaison de mécanismes biotiques et abiotiques.

Received December 13, 1991
Accepted September 3, 1992
(JB331)

Reçu le 13 décembre 1991
Accepté le 3 septembre 1992

Knowledge of factors that regulate nitrogen flux in streams is integral to our understanding of lotic structure and function (Meyer et al. 1988). Studies over the last decade have greatly enhanced our knowledge of nitrogen transport in a variety of lotic systems both temperate and tropical (Vitousek et al. 1979; Meybeck 1982; Newbold et al. 1983; Grimm and Fisher 1984, 1989; Lesack et al. 1984; Sebetich et al. 1984; Richey et al. 1985; Grimm 1987; Saunders and Lewis 1988; Triska et al. 1989a; Munn and Meyer 1990; Webster et al. 1991). The combination of abiotic conditions that influence nitrogen flux in tropical streams can be quite different from their temperate counterparts. For instance, tropical lowland rain-forests on Costa Rica's Atlantic slope receive high annual precipitation (4 m) and lack seasonality in temperature, precipitation, and detrital input. Streams draining primary forest are also severely light limited due to dense multistratal canopies.

While some information exists regarding nitrogen transport in New World tropical streams draining highly weathered soils of the Amazon (Lewis 1986), virtually nothing is known about nitrogen flux in streams of Central America. Central American landscapes can be of geologically recent volcanic origin (10^2–10^3 yr) and are often underlain by active geothermal systems. Geothermal activity along the volcanic spine of Costa Rica plays a major role in determining patterns of the solute chemistry in surface waters with respect to phosphorus, silica, and major cations and anions such as SO_4, Cl, Mg, and Ca (Pringle et al. 1993).

The Salto River, a stream draining Costa Rica's Atlantic slope, receives groundwater inputs of geothermally based soluble reactive phosphorus (SRP) at a gradient break where the stream drains lowland swamp forest (Pringle et al. 1990; Pringle and Triska 1991). The geothermal origin of solute-rich groundwater entering the stream is indicated by high solute levels (e.g. Cl, Mg, Ca, HCO_3, and SO_4) rather than thermal properties, since temperature cools to that of ambient groundwater during subsurface transport (Pringle 1991; Pringle et al. 1993). SRP input is nearly constant at numerous springs and seeps located in the channel and along the banks; however, channel waters of the Salto range between 50 and 200 μg P/L depending on the magnitude of dilution by recent storm runoff. In contrast, the Pantano tributary is unmodified by geothermal activity, with SRP concentrations typically <10 μg/L.

Nutrient cycles are biologically coupled based on the element ratio required for the production of biomass. Atomic ratios of $N/P > 16$ can indicate phosphorus limitation of primary production whereas $N/P < 16$ can indicate nitrogen limitation (Redfield et al. 1963). The input of SRP to the Salto causes the dissolved inorganic nitrogen (DIN)/SRP ratio within the drainage to vary from as low as 3 in the main stem Salto to as high as 55 in the Pantano tributary under base flow conditions. This wide range in N/P ratios raises the question: does high, natural SRP enrichment influence the retention, transport, and transformation of DIN in otherwise similar lowland, tropical streams? We addressed this question in three phases: first, we characterized ammonium levels in springs, seeps, groundwater, and channel water of both streams and evaluated these levels with respect to $NO_2 + NO_3$-N and SRP data presented in previous studies (Pringle 1991; Pringle and Triska 1991); second, upon finding high ammonium levels in groundwater and high nitrate in stream water of both streams, we assayed two types of channel sediment for nitrification potential using laboratory slurry procedures; third, we separately added ammonium and nitrate to stream reaches to examine the fate of DIN under both high- and low-SRP regimes. We hypothesized effective retention and transformation of ammonium in both streams due to low background concentrations, preference of ammonium over nitrate for biotic uptake, and potential for nitrification in channel sediments. We also hypothesized depletion of background SRP in the Pantano during ammonium amendment due to low SRP concentration, enhanced biotic demand, and lack of significant SRP enrichment from geothermal sources along the reach. Finally, we hypothesized conservative transport of amended nitrate in both streams due to high background concentrations.

Study Site

This study was conducted at La Selva Biological Station, a 3300-ha reserve in northern Costa Rica (10°26′N, 84°01′W) (Fig. 1), located in the transition between the Caribbean lowland plains and the steep foothills of the central mountain range.

The station receives an average annual rainfall of 4 m. Historically, the wettest months are June–August and the driest February–April; however, some years lack a definite dry season.

The Salto River is an ungauged third-order stream that drops from 300 to ~36 m above mean sea level where it drains into the Puerto Viejo River. The base of the watershed is located in La Selva Biological Reserve and the upper two thirds is within Braulio Carrillo National Park, a corridor of land comprising part of the last continuous altitudinal transect of tropical rainforest spanning elevations from sea level to 2900 m that remains on the Atlantic Slope of Central America. More than 95% of the Salto watershed is in primary, tropical rainforest (Pringle et al. 1985).

The lower Salto watershed, where the experimental reaches were located, is composed primarily of terraces of alluvial clays, sands, and gravels of volcanic origin (Bourgeois et al. 1972) whereas the upper watershed is a basalt formation believed to be of Quaternary origin. The Salto mainstem runs along the eastern boundary of the younger (Esquina) of the two lava flows at La Selva (Pringle and Triska 1991). Phosphate-rich springs emerge near the edge of this formation at numerous locations (Pringle 1991). The Esquina flow overlies the older Salto flow and is andesitic, dating from the early Pleistocene. The Salto flow, which is andesitic/basaltic, is believed to have been deposited in the middle of the early Pleistocene (Sollins et al. 1993). The low-phosphate Pantano tributary drains the Salto lava flow.

A previous study of 24 seeps and springs along the Pantano and Salto indicated that SRP concentrations in springs along the Salto ranged from 2 to 260 μg/L but only 5–15 μg/L along the Pantano (Pringle 1991). Eight springs along the Salto exceeded 200 μg/L, the maximum SRP level observed in channel water. Springs of both high and low SRP concentration were found along the Salto, but only low-SRP waters were observed along the Pantano.

The study reach was located in a swamp forest close to the break in landform. Drainage throughout the site is impeded by the bedrock threshold of the low foothills. Soils in the swamp forest (Fig. 1) are poorly drained, blue–grey, and blackish-brown silts and clays. They are slightly acidic (pH 4.0–5.2) and have a relatively high organic content (~25%) and fragile structure (Bourgeois et al. 1972). Bankside soils in upland reaches of both streams are classified as lithic humitropepts, but within the lowland swamp forest, they are typic, histic, or lithic tropaquepts (Sancho and Mata 1987).

Methods

Ammonium, total DIN concentration, and N/P ratio were determined for surface water (Salto) and 24 bankside springs along the Salto and Pantano (Fig. 1). SRP and $NO_2 + NO_3$ data from these sites were previously reported in Pringle (1991). The samples, collected September 1988, were filtered in the field (0.45-μm membrane) into new polyethylene bottles rinsed three times with sample. Ammonium was analyzed at the biological station's laboratory using a phenolhypochlorite method (Solorzano 1969). Surface and groundwater chemistry were also compared along four well transects (April 1987). Three transects were on the Salto (upper swamp and east and west banks of midswamp) and one on the Pantano (Fig. 1). Each

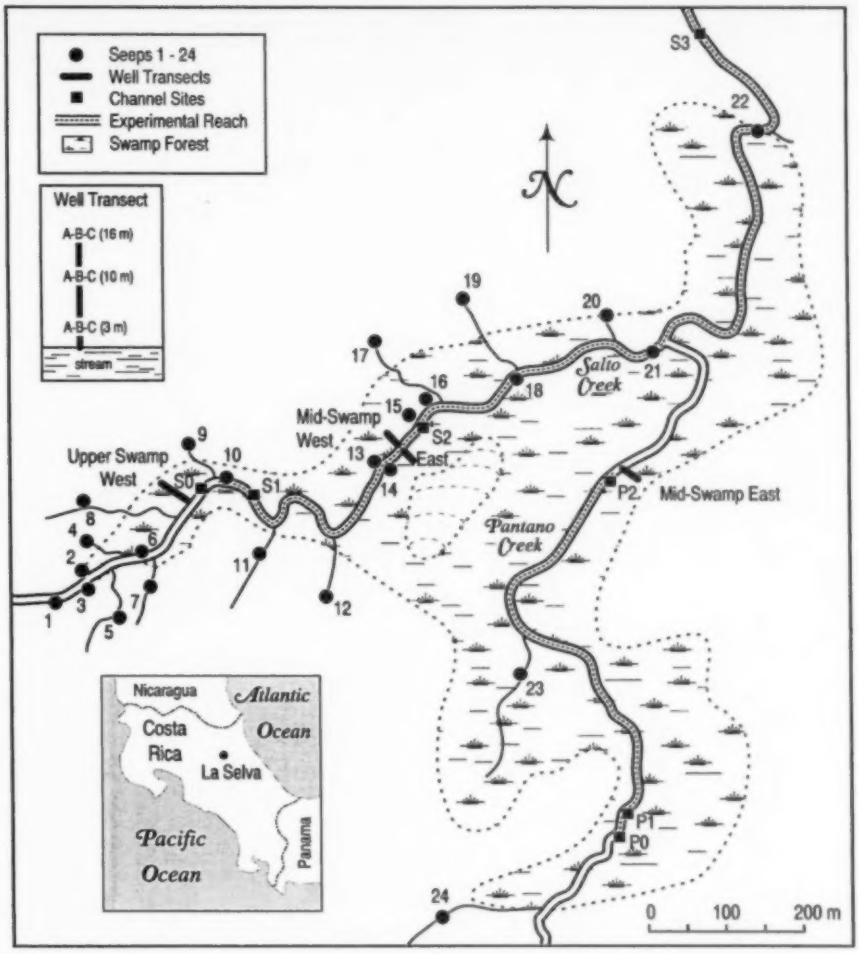

FIG. 1. Map of the Salto and Pantano watershed in Costa Rica showing the location of bankside seeps, groundwater well transects, labeled either west or east, and arrangement of wells along a typical transect. Sample sites for whole-system amendments are labeled S0–S3 along the Salto and P0–P2 along the Pantano (figure modified from Pringle 1991).

transect consisted of two or three well arrays at distances of 3, 10, and 16 m perpendicular to the channel and 1–2 m into the water table. Each array consisted of three wells (A–C) with a minimum of 3 m spacing between wells. Transects were aligned along the anticipated groundwater flow path. Wells were drilled with a power auger and lined with polyvinyl pipe (10 cm) to which a 0.006 wellpoint (slot width of ~130 μm) was attached to the base. Each well was topped with a removable cap. One day prior to sampling, the wells were pumped free of standing water and allowed to refill. The samples were collected using a peristaltic pump connected to an inline filter (0.45-μm membrane filters, Millipore or Gelman metricel) into new polyethylene bottles. Three successive aliquots of sample water were passed through the filter and used to rinse the bottle (as above) before the fourth was saved as the sample. This sampling protocol was also used for water collected in the nutrient amendment studies. The samples were returned to the laboratory and immediately frozen for subsequent analysis.

Nitrification potential of sediments from the Salto was assayed in a 48-h laboratory study (August 1988). Two dominant sediment types, a firm clay and an uncompacted, soft, sandy, organic-rich sediment, were assayed. Six 250-mL flasks of each sediment type contained 20 mL of sediment, 120 mL of stream water, ammonium amended to 1.0 mM as $(NH_4)_2SO_4$, and phosphate to 1.0 mM as K_2HPO_4. Then duplicate flasks of each sediment type were inhibited with nitrapyrin (an inhibitor of ammonium oxidation (10 μL of 22% solution/flask)), heat killed, or left untreated. Water was sampled (10 mL) at 0, 5, 24, and 48 h. Samples were passed through a preleached 25-mm Gelman acrodisc filter (0.45 μm), refrigerated, and analyzed for nitrate within 24 h. Between the taking of samples, flasks were cotton stoppered and shaken to maintain aerobic conditions.

DIN retention–transport was determined under naturally enriched and low-SRP conditions using whole-stream tracer injection techniques (Triska et al. 1989a, 1989b, 1990; Solute

TABLE 1. Dates and background conditions of whole-system manipulations to Pantano and Salto creeks.

Site	Treatment (target concentration)	Date (1989)	Injection time	Background concentration NO_3 (μg N/L)	NH_4 (μg N/L)	PO_4 (μg P/L)	N/P
Salto	Ammonium (100 μg N/L)	Feb. 19	09:00–13:00	188	7	119	3
Pantano	Ammonium (100 μg N/L)	Feb. 20	09:30–12:30	134	18	6	55
Salto	Nitrate (400 μg N/L)	Feb. 22	08:00–11:00	187	6	124	3
Pantano	Nitrate (400μg N/L)	Feb. 24	08:00–11:00	125	22	6	55

Transport Workshop 1990) during February 1989. The method involved injection of a conservative tracer in conjunction with a reactive solute. The tracer was used to estimate hydrologic parameters such as discharge and dilution whereas the biologically reactive solute was used to estimate biological uptake and sorption after correction for hydrologic properties of the channel. Biotically reactive solutes (BRS) included ammonium (NH_4Cl) and nitrate ($NaNO_3$). The tracer was rhodamine WT, a dye commonly used as a hydrologic tracer (Wilson et al. 1984). One solute and the tracer were mixed in a polyethylene tank and pumped into the center of the channel using an FMI (Fluid Metering, Inc.) battery-operated pump (5–58 mL/min capacity). Amendment periods lasted 3–4 h (Table 1). In the Pantano, water samples were collected ~3 m upstream of the injection point (site P0) and at two downstream sites, P1 and P2, located 40 and 600 m downstream of the injection point (Fig. 1). In the Salto, four stations were sampled: ~3 m upstream of the injection point (S0) and three downstream sites, S1 (50 m), S2 (460 m), and S3 (1330 m). Dates and details of each injection are described in Table 1. Nutrient samples were frozen and transported to the U.S. Geological Survey in Menlo Park, California, for analysis. Ambient fluorescence and fluorescence produced by rhodamine WT amendments were determined in unfiltered samples using a Turner III fluorometer immediately upon return to La Selva Biological Station's laboratory. Fractional recovery of amended BRS was calculated for each downstream station by correcting for dilution with the rhodamine WT data:

$$\text{BRS calculated} = \text{BRS}_{up} + \frac{[\text{BRS}_t]}{[\text{Rh}_t]} \cdot [\text{Rh}_d - \text{Rh}_{up}]$$

$$\text{BRS measured} = \text{BRS}_d$$

$$\text{Fraction recovered} = \frac{\text{BRS measured}}{\text{BRS calculated}}$$

where BRS = biologically reactive solute concentration, Rh = rhodamine WT concentration, up = upstream concentration, d = downstream concentration, and t = tank concentration. The difference between median calculated and measured plateau concentrations was compared using a t-test on the signed ranks (Iman and Conover 1983).

Nitrate, ammonium, and SRP in samples collected during the amendment studies were measured on a Technicon Autoanalyzer II as follows: Nitrate-(includes nitrite) by cadmium reduction (Technicon Industrial Method No. 158-71 W, December 1972) and SRP by a molybdenum-blue method (Technicon Industrial Method No. 155-71 W, January 1973). Analytical precision was about 1 μg/L for nitrate nitrogen and SRP below 100 μg/L. At higher levels, SRP and nitrate precision was about 1% of the reported value. The precision of the SRP and nitrate methods was estimated by thawing, analyzing, and refreezing three reference samples repeatedly over a 5-12-mo period. Ammonium-nitrogen was analyzed by a phenol-hypochlorite method (Technicon Industrial Method No. 154-71 W, February 1973). Ammonium precision was about 2 μg/L at all levels encountered in this study. Although no reference-sample study was conducted for the ammonium method, the calibration precision as measured by linear-regression analysis, was approximately the same as that of the other two methods. The standard deviation of the ammonium analysis was thus roughly estimated to be <2 μg N/L for concentrations <120 μg N/L.

Results

Synoptic Sampling

The DIN concentration of seeps and springs ranged from 79 to 213 μg/L and, with one exception (Site 13), nitrate exceeded ammonium concentration (Table 2). At 13 of the 22 Salto locations, ammonium in seeps exceeded that in channel water. Ammonium levels were lowest in seeps and springs of the upper swamp (e.g. Sites 1–5) and highest at midswamp locations (Sites 13–15). At 18 of 22 sites, nitrate levels were lower in seeps than in channel water. With the exception of Site 9 on the Salto and Site 23 on the Pantano, DIN/SRP was <16, indicating SRP sufficiency for biotic production.

Groundwater wells within arrays often exhibited high variation in nutrient concentration (Table 3). However, nutrient concentrations in groundwater and stream water and between transects were sufficiently different, such that distinct trends in nutrient concentration were readily observed. For example, SRP in Pantano groundwater was lower than in Salto groundwater, and SRP in groundwater along the west bank of the Salto was lower than in comparable sites on the east bank. The lowest ammonium concentrations were found in groundwaters of the upper swamp. Ammonium exceeded nitrate in 19 of the 27 groundwater wells, and six of the eight exceptions were in the upper swamp where SRP levels were also low. Groundwater ammonium levels greatly exceeded channel concentrations in the midswamp Salto and the 3-m wells of the Pantano. Groundwater ammonium levels generally decreased with distance from the channel. Nitrate in groundwater was always less than in channel water. A general pattern observed in the synoptic sampling was that ammonium concentration decreased (groundwater > seep water or channel water) whereas nitrate concentration increased (groundwater < seep water < channel water) in both streams.

Sediment Nitrification Potential

The high ammonium concentration in groundwater, intermediate concentration in seep water, and low concentration in

TABLE 2. SRP, nitrate, ammonium and DIN concentrations (μg/L) and DIN/SRP by atoms in seep and spring waters along the banks of Salto and Pantano creeks and in the channel, August 1988. The site numbers correspond to Fig. 1. SRP and nitrite plus nitrate data from Pringle (1991).

Site	SRP	NO_2 + NO_3-N	NH_4-N	DIN	DIN/SRP
Salto					
Upper swamp					
West bank					
1	260	80	2	82	0.7
2	231	55	24	79	0.8
4	208	72	18	90	1.0
6	60	115	64	179	6.6
8	32	55	27	82	5.7
9	2	137	20	157	174.4
10	62	110	18	128	4.6
East bank					
3	57	194	15	209	8.1
5	233	121	12	133	1.3
7	158	171	42	213	3.0
11	34	105	16	121	8.1
Midswamp					
West bank					
13	77	94	122	216	6.2
15	33	82	48	130	8.8
16	64	107	24	131	4.5
17	259	47	40	87	0.7
19	106	53	22	75	1.6
20	25	84	50	134	11.91
East bank					
12	219	74	27	101	1.0
14	222	87	50	137	1.4
18	241	57	30	87	0.8
21	27	50	46	96	7.9
22	66	54	53	107	3.6
Upper Swamp					
Streamwater	51	137	27	164	7.1
Pantano					
Upper swamp					
West bank					
23	<5	212	N/A	N/A	94
24	15	38	N/A	N/A	5.6

channel water suggested that nitrification may occur during subsurface and channel transport. To assay for nitrification potential the two sediment types described earlier were collected from the geothermally modified reach of the Salto where high nutrient levels minimized nutrient limitation. Slurries from both sediment types exhibited nitrate increase, and duplicate flasks had nearly identical rates of production (Fig. 2). Nitrate production with the firmer clay substratum (3.34 $\mu gN \cdot cm^{-3} \cdot d^{-1}$) was nearly twice that of uncompacted organic-rich sediments (1.76 $\mu g\ N \cdot cm^{-3} \cdot d^{-1}$). Both heat killing and nitrapyrin effectively curtailed nitrate production.

Hydrologic Tracer

Although our whole-system amendments were conducted during the dry season, precipitation was common, particularly at night. As a result of the Salto's larger watershed area and the timing of precipitation, discharge varied between experiments in the Salto but not in the Pantano (Table 4). Rhodamine WT dilution indicated that discharge at the downstream station of the Salto (S3) was 544 L/s during the ammonium amendment and 841 L/s during the nitrate amendment. The Pantano contributed only 35 L/s, less than 10% of the total flow calculated at S3. This is a minimum estimate because the base of the experimental reach, where discharge was calculated, was a significant distance upstream of the confluence (Fig. 1). Discharge within the experimental reach in the Salto increased downstream by 216 L/s on February 19 and by 421 L/s on February 22. This increase, while heavily dependent on recent runoff, also reflected the contribution of seeps, springs, and groundwater inputs. The capacity for nutrient retention was related to both the size of the reach (width and length) and the potential contact time with channel sediments. High discharge reduced the nominal travel time for nutrient transport by 40 min between the two experiments in the Salto whereas travel times were nearly identical for the Pantano experiments.

Ammonium Injection

Amendment into the Pantano raised the ammonium concentration to approximately 100 μg N/L. Measured median plateau concentration at P1 was 104 μg N/L between 09:54 and 12:32 (Fig. 3; Table 5). The median plateau concentration downstream (P2) between 14:40 and 16:00 was 42 μg N/L. The

TABLE 3. Dissolved inorganic nitrogen and phosphorus concentrations (μg/L) during a synoptic sampling of groundwater wells, bankside seeps, and channel water, April 1987. Each datum is a mean of duplicate samples. Well names and locations are according to Fig. 1 NS = no sampling. Table modified from Pringle and Triska (1991).

	Salto									
			Midswamp							
	Upper swamp West bank		West bank			East bank		Pantano Midswamp East bank		
	3 m	10 m	3 m	10 m	16 m	3 m	10 m	3 m	10 m	
					Phosphate					
Well										
A	15	75	30	7	12	96	99	7	15	
B	37	39	78	75	14	183	93	4	6	
C	24	43	15	88	1	235	19	4	3	
Seep	20			200		227		NS		
Channel	127				139			20		
					Ammonium					
Well										
A	11	14	527	357	215	18	10	685	26	
B	13	11	212	537	165	22	27	947	52	
C	22	11	324	167	213	28	18	140	120	
Seep	22			12		11		NS		
Channel	NS				15			50		
					Nitrate					
Well										
A	13	63	5	3	3	4	18	15	88	
B	28	66	13	16	7	2	10	6	2	
C	47	113	6	25	2	2	3	3	5	
Seep	78			120		136		NS		
Channel	167				165			193		

TABLE 4. Estimates of discharge and nominal travel time in experimental reaches of the Salto and Pantano creeks.

Salto			Pantano		
Date (1989)	Discharge (L/s)	Nominal travel time (min)	Date	Discharge (L/s)	Nominal travel time (min)
Feb. 19			Feb. 20		
S1	328	20	P1	21.7	12
S2	392	85	P2	35	240
S3	544	310			
Feb. 22			Feb. 24		
S1	420	<10	P1	21.1	15
S2	573	50	P2	36.9	230
S3	841	270			

measured concentration was significantly lower than that calculated by rhodamine dilution (95% confidence limits) at both P1 (15.0 μg N/L) and P2 (39 μg N/L) (Table 5). Under plateau conditions, only 36% of upstream ammonium was recovered at P2, indicating significant retention during transport.

Nitrate concentration rose as the ammonium pulse was transported down the Pantano (Fig. 4). Prior to injection, nitrate concentration at P2 averaged 133 ± 3 μg N/L (± SD) but rose to 151 ± 5 μg N/L at the ammonium plateau concentration. The maximum nitrate concentration was 155 μg N/L. The nitrate production of approximately 20 μg/L accounted for approximately 50% of the 39 μg N/L difference between measured and calculated ammonium concentration at P2. The rise, peak, and fall of nitrate concentration lagged that of ammonium

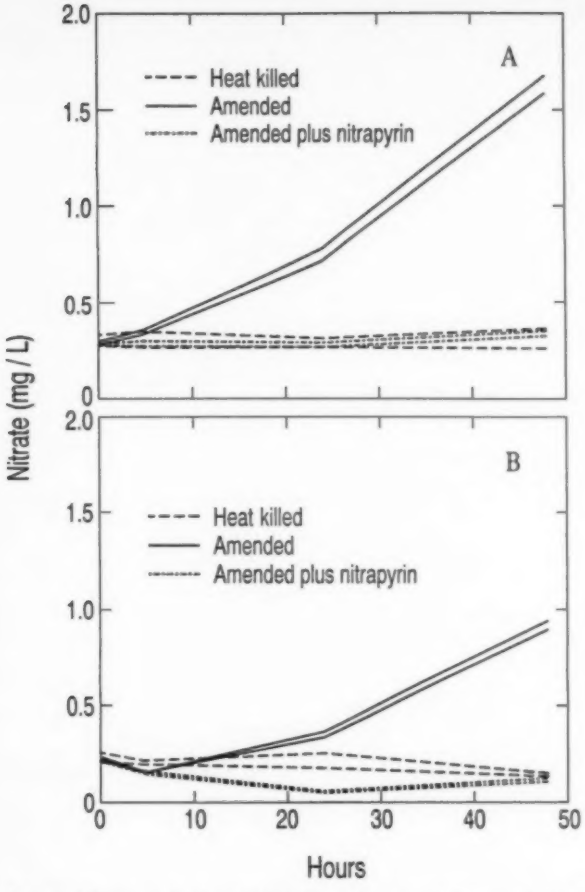

FIG. 2. Nitrate production in (A) clay sediment and (B) organic-rich sediment slurries in response to 1.0 mM ammonium and phosphate amendment.

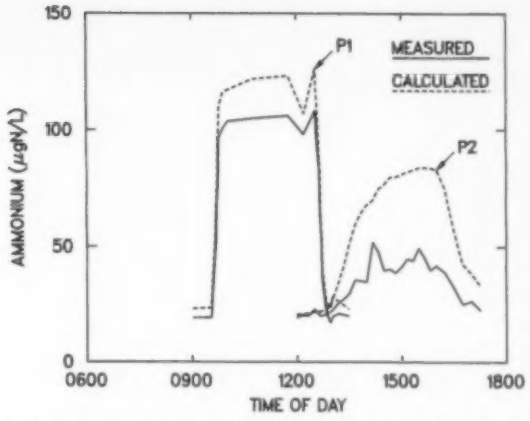

FIG. 3. Comparison of measured concentration of ammonium to that calculated from coinjected rhodamine WT to correct for dilution–dispersion during a whole-system amendment to a reach of Pantano Creek.

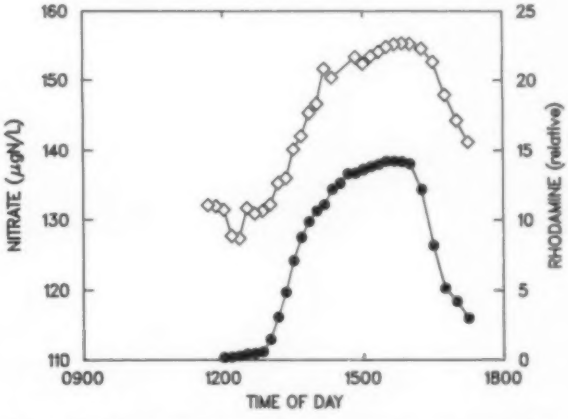

FIG. 4. Timing of nitrate concentration increase (open symbols) at Pantano site P2 in response to ammonium plus tracer amendment as indicated by the conservative tracer plume (solid symbols).

by approximately 30 min. Background SRP was 6.2 µg P/L at P0 and 9.2 µg P/L at P2 and did not change significantly during the experiment.

Ammonium was amended to the Salto at the same target concentration as the Pantano (Fig. 5). Median measured ammonium concentration was 114 µg N/L during the plateau at S1 (09:15–13:05) compared with a median calculated concentration of 119 µg N/L. Median measured and calculated ammonium levels at the midreach station (S2) during plateau were reduced to 95 and 108 µg N/L, respectively (11:40–14:03). At S3, median measured and calculated ammonium levels under plateau conditions were 65 and 86 µg N/L, respectively. The 21 µg N/L difference is significant, indicating effective ammonium retention. Nitrate concentration changed only slightly between S1 and S2 during the ammonium amendment. Between S2 and S3, however, the results were similar to the Pantano in that nitrate concentration increased as the ammonium pulse was transported down the channel (Fig. 6). Prior to arrival of the ammonium pulse at S3, nitrate averaged 170 ± 1 µg N/L but rose to 180 ± 1 µg N/L as ammonium reached plateau concentration. As in the Pantano, the 10 µg/L increase in nitrate during the ammonium pulse was approximately 50% of the 20 µg/L difference between measured and calculated ammonium concentrations at S3. Maximum nitrate concentration was 183 µg N/L. SRP decreased from 119 to 104 µg P/L between S0 and S3 but exceeded that predicted by dilution. Continuous SRP input masked any detectable response to the ammonium amendment.

Nitrate Injections

Nitrate added to the Pantano raised the measured median concentration from 125 to 464 µg N/L at P1 (08:18–11:07) (Fig. 7; Table 5). Median calculated concentration was 497 µg N/L. This difference in median concentrations was significant, indicating nitrate loss between P0 and P1. Measured median concentration at the downstream site (P2) during plateau (13:00–14:30) was 347 µg N/L, 75% of the upstream concentration. Median calculated nitrate concentration was 334 µg N/L at P2. The difference between median measured and calcu-

TABLE 5. Measured concentration (mean and median) of ammonium or nitrate at plateau concentration during their respective amendments, and the median difference between measured and calculated concentrations, 1989. Positive median differences indicate disappearance in excess of dilution. Negative differences indicate production. The *P*-value indicates the statistical significance of the difference between median measured and calculated concentrations. P = Pantano, S = Salto, *n* = number of observations at plateau concentration, SD = standard deviation.

Date (1989)	*n*	Mean concentration (μg N/L ± SD)	Median concentration (μg N/L)	Median difference (μg N/L)	*P*-value
		Ammonium amendment			
Feb. 20					
P1	6	104 ± 4	104	15.0	0.036
P2	9	43 ± 3	42	39.0	0.009
Feb. 19					
S1	10	113 ± 4	114	6.0	0.008
S2	15	95 ± 2	95	11.7	0.001
S3	13	66 ± 2	66	20.1	0.002
		Nitrate amendment			
Feb. 24					
P1	9	463 ± 13	464	31.7	0.009
P2	10	343 ± 12	347	−12.4	0.032
Feb. 22					
S1	7	565 ± 14	567	18.8	0.022
S2	12	465 ± 9	482	−4.0	0.005
S3	6	393 ± 5	396	−16.8	0.003

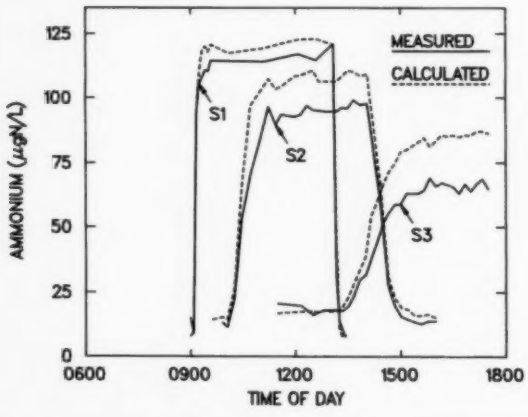

FIG. 5. Comparison of measured concentration of ammonium with that calculated from coinjected rhodamine WT to correct for dilution–dispersion during a whole-system amendment to the Salto River. S1, S2, and S3 are sample sites (see Fig. 1).

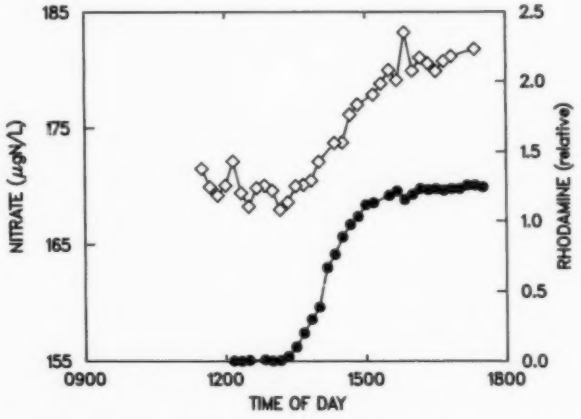

FIG. 6. Timing of nitrate concentration increase (open symbols) at Salto site S3 in response to ammonium plus tracer amendment as indicated by the conservative tracer plume (solid symbols).

lated nitrate was significant, indicating nitrate gain during passage from P1 to P2. Background SRP increased from 5.3 μg P/L at P0 to 9.3 μg P/L at P2 and exhibited no significant response to nitrate amendment.

Nitrate added to the Salto raised the median measured concentration from 189 to 567 μg N/L at S1 (08:18–09:30) (Fig. 8). Median measured (567 μg N/L) versus calculated concentrations (584 μg N/L) were significantly different, indicating nitrate retention between S0 and S1 (Table 5). The opposite was observed between S2 and S3. Between S2 and S3 the difference in median measured (396 μg N/L) and calculated concentrations (377 μg N/L) was significant, indicating nitrate gain. This represents the minimum amount of nitrate production, since background concentration decreased by approximately 12 μg N/L between S2 and S3 prior to passage of the nutrient pulse. Mean SRP concentration decreased downstream from 123 μg P/L at S0 and S1 to 117 μg P/L at S2 and 107 μg P/L at S3. SRP concentrations remained steady during the passage of the nitrate pulse. Thus, nitrate amendment to the Salto did not affect SRP concentration.

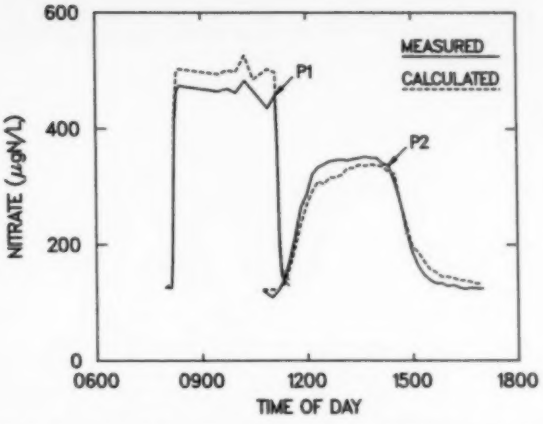

FIG. 7. Comparison of measured concentration of nitrate with that calculated from coinjected rhodamine WT to correct for dilution–dispersion during a whole-system amendment to a reach of Pantano Creek. P1 and P2 are sample sites (see Fig. 1).

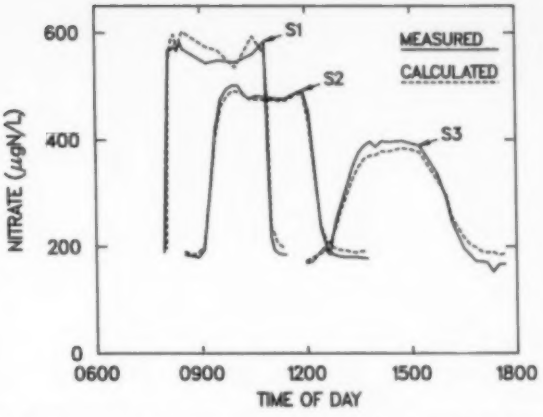

FIG. 8. Comparison of measured concentration of nitrate with that calculated from coinjected rhodamine WT to correct for dilution–dispersion during a whole-system amendment to a reach of the Salto River. S1, S2, and S3 are sample sites (see Fig. 1).

Discussion

While geothermal SRP enrichment of the Salto River constituted a second, separate phosphate source to surface water, DIN in both streams appeared to result from mineralization in the catchment. Only small differences in DIN occurred between the Salto and Pantano in contrast with SRP. Geothermally based groundwaters, high in SRP, appeared to be low in DIN. On the east Salto, especially between midswamp and the confluence of the Salto and Pantano, the drainage is small and entirely swamp forest whereas the west Salto has an extensive contributing area consisting of both upland forest and swamp forest. Where local drainage is aerially extensive (i.e. upper swamp and west Salto transects) or where geothermal groundwater input is minimal (i.e. Pantano), groundwater DIN concentration was high. In the Salto's east bank, where the contributing area of swamp forest is small and groundwater is heavily impacted by geothermal sources, SRP was high but DIN was low. Geothermal waters can be high in ammonium, especially near their source (Cooper 1983). However, the low concentration in the east bank of the Salto indicated absence of a significant geothermal DIN contribution.

DIN composition along both streams reflected local redox conditions. Seep water and groundwater of the Salto's upper swamp were typically oxic (Pringle and Triska 1991). Concentrations of nitrate were higher and ammonium lower than midswamp. Midswamp groundwaters with low dissolved oxygen concentration (<1.0 mg/L) were often found along the west and east transects of the Salto (3-m Wells). At these sites, DIN was dominated by ammonium.

Higher ammonium than nitrate concentration in groundwater and higher nitrate than ammonium concentration in stream water indicated nitrification potential during ammonium transport. Nitrate production in sediment slurries and lack of formation in killed and nitrapyrin-inhibited treatments suggested that nitrate production was a consequence of biological nitrification. Sediments from the Salto had potential nitrification rates of 1.76 μg $N \cdot cm^{-3} \cdot d^{-1}$ for organic-rich sediments to 3.34 μg $N \cdot cm^{-3} \cdot d^{-1}$ for clay sediments. Nitrification has also been reported in soils at La Selva. Robertson (1984) found soil rates of 4 μg $N \cdot g^{-1} \cdot d^{-1}$ which are at the high end of the range of nitrifying activity (<0.1 to <6.0 μg $N \cdot g^{-1} \cdot d^{-1}$ for tropical soils (de Rham 1970; Tanner 1977; Lamb 1980; Vitousek et al. 1983).

Comparison between the SRP-enriched Salto and the unmodified Pantano under nutrient-amended conditions indicated similarities in nitrogen retention, transformation, and transport. Ammonium recovery at the base of the reach (corrected for background and dilution) averaged only 37% of upstream input in the Pantano and 70% in the Salto, indicating significant retention or transformation during downstream transport. Ammonium amendment resulted in increased nitrate concentration at the base of the experimental reach in both streams. The increase in nitrate concentration accounted for approximately 50% of the difference between mean measured and mean calculated ammonium concentration in both streams. Timing of the ammonium pulse, indicated by rhodamine WT (Fig. 5 and 7), suggests that nitrification in the channel is a reasonable explanation for rising nitrate concentration during the whole-system amendments. Nitrifying activity during ammonium injections to surface water (Newbold et al. 1983; Richey et al. 1985) and hyporheic water (Triska et al. 1990) has also been observed in temperate streams.

We hypothesized SRP depletion in the Pantano due to assimilatory ammonium demand and lack of geothermal SRP enrichment. If the calculated ammonium retention of 39 μg N/L at P2 (Table 5) was exclusively assimilated for biomass production, and coupled to SRP uptake at a ratio of 16/1, SRP concentration would have been depleted by 5.4 μg/L, more than half the background concentration. However, SRP depletion was not observed. There are several explanations. First, while nitrification is coupled to carbon fixation, and SRP uptake might be expected, the ratio of ammonium oxidized to carbon fixed by nitrifying bacteria is higher than the Redfield ratio of 106C:16N:1P (Redfield et al. 1963). Alexander (1961) provided N:C ratios (ammonium-nitrogen oxidized to carbon fixed) of 35:1 for *Nitrosomonas europaea* and N:C ratios from 14:1 to 70:1 for other ammonium-oxidizing species. Thus, nitrification, a major fate of amended ammonium, oxidized large amounts of ammonium for the amount of carbon fixed

and generated little SRP demand. Second, an unknown increment of retention was sorption rather than uptake. Ammonium is readily sorbed to clays and organic matter (Boatman and Murray 1982), and both are important constituents of bed sediments in the Salto and Pantano. Third, background SRP concentration in the Pantano may be controlled by abiotic rather than biotic processes. Mulholland et al. (1990), studying a small forested stream, found that biologically controlled SRP uptake mechanisms became saturated above approximately 5 μg P/L whereas physical–chemical adsorption continued to increase with increasing concentration. Pringle and Triska (1991) proposed that abiotic control of SRP levels in the Pantano after SRP amendment to the water column (36 ± 2 μg P/L) was effectively reduced to background concentration with no DIN response. Finally, mineralogically identical soils adjacent to the Salto have far more leachable SRP than at the Pantano, presumably indicating availability of additional sorption sites.

Bioassays (15 d) using nutrient-diffusing substrata produced a significant chlorophyll response to SRP in light gaps along the Pantano, but not in the Salto (Pringle and Triska 1991). Thus, ambient SRP level may impact biotic uptake by autotrophs, but biotic uptake does not have a large impact on stream water SRP levels in either stream, most likely due to the high density of shading.

Our final hypothesis was that amended nitrate would be transported conservatively in both streams due to high background concentration. Not only was nitrate transported conservatively in both the Salto and Pantano, but a small increase in nitrate concentration was observed at the base of each reach. The mechanism for this increase is unknown. Previous 15-d bioassays found no significant increase in chlorophyll *a* accrual rates in either the Salto or Pantano using nutrient-diffusing substrata amended with nitrate (Pringle et al. 1986; Pringle and Triska 1991). Conservative to nearly conservative nitrate transport has commonly been reported in streams. Webster et al. (1991) found significant nitrate uptake in only 11 of 58 experiments conducted at Coweeta Hydrologic Laboratory, North Carolina. Richey et al. (1985) reported conservative transport of nitrate but effective retention of ammonium and urea at Bear Brook, New Hampshire. High nitrate retention within short stream reaches is most often attributed to high autotrophic production (Sebetich et al. 1984; Grimm 1987; Triska et al. 1989a, 1989b), although detritus-associated uptake (Munn and Meyer 1990) and denitrification (Hill 1988) can also produce measurable nitrate retention. In the Salto and Pantano, biotic demand was insufficient and uptake length too great for nitrate retention to be detected by our methods.

Our study effectively characterized sources and the fate of transported DIN under naturally high- and low-SRP conditions in tropical streams draining parent material of relatively recent volcanic origin. We found that the proportion of nitrate to ammonium in upper and midswamp locations reflected local redox conditions. The pattern of ammonium concentration was groundwater > spring water > channel water whereas the nitrate pattern was the opposite. Nitrification in channel sediments was confirmed by slurry studies and whole-system amendments. Ammonium retention and transformation were high during down-channel transport. Nitrate production exceeded retention under both nitrate and ammonium amendment. DIN retention, transport, and transformation were not related to SRP levels. Absence of an SRP response to either amendment in the Pantano may indicate a limited role for DIN assimilation compared with sorption and nitrification. The results support our previous conclusion (Pringle and Triska 1991) that abiotic factors regulate SRP concentration in the Pantano. However, biotic and abiotic mechanisms are both important in regulating DIN concentration and composition. These observations provide an important base for future controlled studies of sediment and biota to examine underlying mechanisms of SRP and DIN retention, transformation, and transport.

Acknowledgements

This study was supported by National Science Foundation grant BSR-91-07772 to C. M. Pringle and F. J. Triska, grant BSR-87-17746 to C. M. Pringle, and to the Jessie Smith Noyes Foundation through the Organization for Tropical Studies which provided funds for well installation and sampling. We thank Rodolfo Vargas-Ramirez and Gregory Browder for assistance in field and laboratory work and the Organization for Tropical Studies for infrastructual support. Use of product names is for identification only and does not constitute endorsement by the U.S. Geological Survey.

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Mirror-Elicited Agonistic Behaviour and Body Morphology as Predictors of Dominance Status in Juvenile Coho Salmon (*Oncorhynchus kisutch*)

L. Blair Holtby, Douglas P. Swain,[1] and G. Michael Allan[2]

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Holtby, L. B., D. P. Swain, and G. M. Allan. 1993. Mirror-elicited agonistic behaviour and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 50: 676–684.

We tested whether body morphology and aggressive behaviour, measured through mirror image stimulation (MIS), could predict the eventual dominance status of juvenile coho salmon (*Oncorhynchus kisutch*) tested in paired contests and in stream tank interactions with several individuals. Dominance in paired contests was predicted by MIS behaviour on the fifth observation day using newly emerged juveniles and by MIS behaviour on the first observation day using 7-wk-old juveniles. In both trials, deeper bodied fish tended to be dominant. In the stream tank tests, the MIS behaviour SAM (swim-against-mirror) and fork length were the best predictors of dominance. Large fish with high levels of SAM tended to be dominant. After removing the effects of fish size, neither body depth nor fin size consistently predicted dominance ability. SAM was positively correlated with overt aggressive behaviours in stream tanks but not with lateral displays. Juvenile coho salmon appeared to react to conspecifics and to their own mirror images in a quantitatively similar way, and both MIS behaviour and body morphology were significant predictors of the outcome of agonistic interactions among individuals.

Nous avons tenté de savoir si la morphologie et un comportement agressif, mesuré par stimulation par l'image dans un miroir (SIM), permettaient de prévoir le rang de dominance de cohos juvéniles (*Oncorhynchus kisutch*) soumis à des essais de comparaison par paires et mis en présence de petits groupes dans des bassins à circulation d'eau. Dans les essais de comparaison par paires, la dominance a été prévue à partir du comportement SIM à partir de la cinquième journée d'observation de juvéniles qui venaient de quitter le fond, ainsi que par le comportement SIM lors de la première journée d'observation de juvéniles de 7 sem. Dans les deux genres d'essais, la dominance paraissait associée à la hauteur du poisson. Dans les essais effectués dans les bassins à circulation d'eau, le fait d'attaquer le miroir et la longueur à la fourche constituaient les meilleurs facteurs de prévision de la dominance. Les gros poissons qui avaient fortement tendance à attaquer le miroir tendaient à être dominants. Après avoir supprimé les effets associés à la taille du poisson, ni la hauteur du corps ni les dimensions des nageoires ne permettaient de prévoir de manière uniforme la capacité d'affirmer la dominance. Il existait une corrélation positive entre l'attaque du miroir et un comportement carrément agressif chez les sujets placés dans les bassins à circulation d'eau, mais dans l'autre type d'essai. Les cohos juvéniles semblaient réagir à des congénères et à leur propre image dans un miroir de manière quantitativement semblable, et le comportement SIM ainsi que la morphologie constituent des facteurs de prévision significatifs du résultat de comportements agonistes.

Received March 9, 1992
Accepted August 26, 1992
(JB429)

Reçu le 9 mars 1992
Accepté le 26 août 1992

Body morphology, level of aggressiveness, and extent of stream residence are correlated between ecological types of coho salmon (*Oncorhynchus kisutch*) (Swain and Holtby 1989), among life-history types of chinook salmon (*Oncorhynchus tshawytscha*) (Carl and Healey 1984; Taylor and Larkin 1986; Taylor 1988), and among the species of *Oncorhynchus* (e.g. Hoar 1951, 1958; Stein et al. 1972; Scott and Crossman 1973). A deeper body and longer, more distinctively coloured dorsal and anal fins are associated with increased aggressiveness and longer stream residence. Fitness in stream-dwelling salmonids may depend on success in aggressive interactions (i.e. on dominance ability; e.g. Chapman 1966). Swain and Holtby (1989) suggested that there might be a functional connection between body morphology and success in aggressive interactions. Their speculation was based on observed morphological and behavioural differences between ecological types of coho salmon. In this study, we test for *within*-population effects of individual differences in body morphology and aggressiveness on agonistic performance (dominance ability).

Understanding the importance of body morphology and aggressiveness to dominance is important in predicting the impacts of artificial propagation of some Pacific salmonids on wild populations. Wild production of coho salmon is supplemented by large hatcheries operating throughout the North Pacific. Hatchery populations can diverge genetically and phenotypically from surrounding wild populations (Greene 1952; Vincent 1960; Flick and Webster 1964; Moyle 1969;

[1]Present address: Department of Fisheries and Oceans, Science Branch, P.O. Box 5030, Moncton, N.B. E1C 9B6, Canada.
[2]Present address: Biology Co-operative Program, University of Victoria, P.O. Box 1700, Victoria, B.C. V8W 2Y2, Canada.

Reisenbichler and McIntyre 1977; Ryman and Stahl 1980; Bachman 1984; Swain and Riddell 1990). Hatchery and wild populations of coho salmon differ in both body morphology (Taylor 1986; Fleming and Gross 1989; Swain et al. 1991) and aggressiveness (Swain and Riddell 1990). Hatchery releases of coho salmon exceed wild production from adjacent systems several-fold in many regions of western North America, and concerns about the genetic effects of straying hatchery fish on wild populations are growing (Ryman and Stahl 1980; Larkin 1981; Simon et al. 1986; Hindar et al. 1991; Saunders 1991). Evidence of increased aggressiveness in hatchery fish (Moyle 1969; Swain and Riddell 1990), although limited, is of particular concern. Aquaculturists are concerned because increased aggressiveness in hatchery fish could indicate that selection for rapid growth has selected for aggressiveness rather than increased growth efficiency (Weatherly 1976). Of more concern are the potential effects of the interbreeding of straying hatchery fish with wild populations. Because aggression is thought to play a central role in population regulation and dispersal (Chapman 1966), aggressive hatchery fish could displace wild fish and reduce production from wild systems (Hindar et al. 1991). Furthermore, there are considerable interactions between coho and other salmonids (Hartman 1965; Glova 1986), and increases in the aggressiveness of coho could lead to adverse effects on other species.

To assess these potential risks of hatchery production, protocols for the measurement of aggressiveness need to be developed. Mirror image stimulation (MIS) tests (Gallup 1968) are quantitative, permit adequate replication, and have been widely used with fishes, including Pacific salmon (Taylor and Larkin 1986; Rosenau and McPhail 1987; Swain and Holtby 1989; Swain and Riddell 1990). MIS tests are subject to the criticism of being unrepresentative of natural interactions because they do not allow the expression of all components of the interactive repertoire. Furthermore, the behaviours seen in MIS tests may be altered or exaggerated compared with those seen in interactions with real opponents (Gallup and Capper 1970; Dore et al. 1978). Nonetheless, MIS behaviours adequately predict between-population differences in agonistic behaviour in coho and chinook salmon. In both species, differences between populations in aggressiveness are in the same direction in MIS tests, paired contests, and group tests (Taylor and Larkin 1986; Rosenau and McPhail 1987; Swain and Holtby 1989; Swain and Riddell 1990). In this study, we use MIS behaviours as measures of aggressiveness in comparisons among individuals *within* populations and examine whether MIS behaviours provide a useful index of aggressiveness in within-population comparisons.

Materials and Methods

We describe results of two sets of experiments. In one set, morphology and MIS behaviour of laboratory-reared juveniles are compared with dominance ability in contests between individuals paired in small aquaria (referred to as the "paired contest" experiments). In the second set, morphology and MIS behaviour of wild-caught juveniles are compared with dominance ability in interactions among several individuals in a stream tank (referred to as the "stream tank" experiments).

In both sets of experiments the MIS measurements were made on fish isolated in 14-L aquaria (30 × 20 × 23 cm deep) with flow-through water exchange (5–15 $mL \cdot s^{-1}$). The aquaria were opaque on three sides and contained 2 cm of fine gravel. The tops of the aquaria were covered with white plastic board. The transparent fronts were covered with black plastic board hoods with small slits through which the fish could be observed. Temperatures were held at levels close to those found at the source sites during the experiments. A 16 h light : 8 h dark illumination schedule was maintained with 40-W fluorescent bulbs arrayed singly behind the tanks. The paired contest experiments were done in the individual aquaria.

The stream tank experiments were done in two 90 × 29 × 25 cm deep sections within an oval Plexiglas stream tank. The bottom of each section was covered with 2–3 cm of fine gravel. Water depth was 20 cm and a water pump maintained a nonturbulent flow of 5–10 $cm \cdot s^{-1}$. In each section, two pieces of shallow aluminum channel were attached to opposite sides of the inside of the tank about 30 cm from the upstream screen. A piece of weighted, white plastic board cut to fit the channel was hung from the ceiling on a pulley. The observer could release the cord and drop the board to the bottom of the tank, quickly isolating the front third of the section. The fish in the front section could then be quickly removed with minimal disturbance to the remaining fish. Water temperatures and the illumination schedule were the same as those in the individual aquaria. The stream tank was visually isolated from the laboratory by ceiling-high black plastic sheeting. Observation slits were cut at tank level.

Fish were fed ad libitum near the end of every day with crushed Tetramin, a commercial flake food, and with brine shrimp nauplii.

Subjects

Paired contests

Between 13 December 1988 and 6 January 1989, gametes were stripped from 10 female and 13 male coho salmon spawning in Mesachie and Halfway creeks in the Cowichan River drainage (Swain and Holtby 1989). Eggs were fertilized in the laboratory to produce 28 full-sib families. Embryos were incubated in Heath trays at 5°C for 375°d and at 8°C thereafter. Between 6 and 30 April 1989, 30–45 alevins from each family were placed in 35-L tanks (1 tank per family) at 8°C. These laboratory-reared juveniles were used in two paired contest trials. For the first trial, 64 fish (1–5 fish per family) were isolated in individual aquaria 2 d after being placed in the 35-L tanks. For the second trial, 54 fish were isolated about 48 d after being placed in the 35-L tanks. Because the latter fish had interacted for more than 6 wk, we term them "socially experienced." Measurement of MIS behaviours began after 5 d of acclimation in the individual aquaria. Fish sizes (mean fork length (FL) ± SD) were 37.0 ± 1.45 mm in trial 1 and 49.0 ± 3.52 mm in trial 2.

Stream tank tests

Coho salmon parr were collected from Bings Creek in the Cowichan River drainage (Fielden and Holtby 1987). On 9 July 1990, two groups were collected from upper Bings Creek (Bings.1 and Bings.2), and on 2 August 1990, a third group (Bings.3) was collected from a site on lower Bings Creek, approximately 1 km downstream of the first collection site. Fish were captured from shallow pools using fine-meshed pole or beach seines. Approximately 200–500 fish were collected at each site, lightly anesthetized in 2-phenoxyethanol, and measured to within 0.5 mm. Approximately 25 fish of the same size (±2 mm) were selected at each site (two groups of different sizes were selected during the first collection), transported to

TABLE 1. Agonistic behaviours recorded in the MIS and stream tank experiments. SLM was recorded only in 1990. "Type" indicates whether a duration (D) or frequency (F) was recorded. Durations were recorded as seconds per 3-min session for MIS experiments or per 10-min session for the sream tank experiments.

Behaviour	Acronym	Type	Definition
Lateral display type 1	LAT1[a]	D	Dorsal and anal fins fully erect with pectoral fins lowered, mouth open and body stiff; animal usually oriented parallel to mirror; often accompanied by paddling with pectorals parallel to mirror in an exaggerated stiffened posture; when motionless, often accompanied by quivering; corresponds to the lateral threat of Chapman (1962) and Hartman (1965) and includes the wigwag of North (1979)
Lateral display type 2	LAT2[a]	D	Distinct from LAT1 in that the dorsal fin is not fully erect and displays are usually motionless but can be accompanied by quivering or swimming in an exaggerated motion parallel to the mirror; LAT2 displays often precede LAT1
Lateral display	LAT[b]	D	LAT1 + LAT2. LAT1 and LAT2 were not distinguished in the stream tank experiments
Swim against mirror	SAM[a]	D	Fish swims, often vigourously, with its snout against the mirror (Taylor and Larkin 1986); often accompanied by nips directed at the image
Swim along mirror	SLM[a]	D	Fish swims vigourously along the mirror with its snout at least partially into the mirror; episodes of SLM and SAM behaviours are often intermixed; not recorded in 1989
Submissive display	SUB[a]	D	Dorsal fin fully depressed with anal fin usually depressed and caudal fin folded; fish is motionless often on bottom or near the surface
Charge	CHRG[c]	F	Rapid, direct and unambiguous motion toward another fish (Kratt and Smith 1979)
Approach	APPR[c]	F	Any other movement by the dominant toward another fish, usually by sideways and backward drifting
Chase	CHSE[c]	F	Pursuit of another fish following an approach or charge, resulting in the displacement of the fish from its original position
Nip	NIP[c]	F	Any biting motion made by the dominant toward another; includes cases without contact between fish, i.e. the "threat nip" of Chapman (1962)
Overt aggression	OVAGGR[a]	D	SAM + SLM (MIS)
	OVAGGR[c]	F	Charge + approach + chase + nip (stream tank)
Aggressive behaviours	AGGR[a]	D	LAT + OVAGGR
Total behaviours	TOTBEHAV[a]	D	AGGR + SUB

[a]MIS only.
[b]Both MIS and stream tank experiments.
[c]Stream tank experiments only.

the laboratory in chilled coolers, and held overnight in 190-L tanks maintained at the temperature of the capture site. The day after capture, each fish was anesthetized in 2-phenoxyethanol, placed on a flat surface and photographed for morphological analysis, uniquely cold-branded (Everest and Edmundson 1967) on the left side, and placed in an individual aquarium for MIS testing. Photography and branding typically required less than 15 s per fish. Fish could be unambiguously identified within 2 d of branding. The fish were allowed to acclimate in the individual aquaria for 5–7 d before the MIS testing began. Fish sizes (mean FL ± SD) were 40.4 ± 1.11 mm (Bings.1), 50.4 ± 1.13 mm (Bings.2), and 63.0 ± 2.51 mm (Bings.3).

Mirror Image Stimulation Experiments

An experimental session began by placing a 20 × 20 cm flat mirror at the back of an aquarium. The mirror entirely occupied the rear face of the aquarium and the fish could see their image from anywhere in the aquarium. After 1 min of unobserved acclimation, the durations of four (1989) or five (1990) distinct behaviours (Table 1) were recorded with stop watches for 3 min. This procedure was repeated for 5 d consecutively. Experimental sessions began at approximately 08:00 every day. Fish were fed after the conclusion of all observations.

For the 1989 experiments using laboratory-reared juveniles, MIS behaviour on each of the five observation days was compared separately with dominance ability. For the 1990 experiments using wild-reared juveniles, MIS behaviour summed over the five observation days was compared with dominance ability. Procedures differed between the two years because of differences in the pretesting history of the experimental subjects. An initial period to develop agonistic behaviour was expected for the newly emerged, socially naive fish used in the first trial in 1989 but not for the socially experienced wild parr used in 1990 (see Discussion).

Dominance Ability

Paired contests

In 1989 at the conclusion of the MIS testing, fish of similar size but different levels of aggressiveness (average AGGR durations) were paired (see Table 1 for acronyms). In the first trial with recently emerged juveniles, FL varied by 0.5 mm or less in 30 pairs and 1.02–2.5 mm in 2 pairs. In the second trial with older juveniles, FL varied by 0.5 mm or less in 16 pairs, 1.0–1.5 mm in 6 pairs, 2.0–2.5 mm in 3 pairs, and 3.5–5.0 mm in 2 pairs. Fish were anesthetized in 2-phenoxyethanol, measured (FL), cold-branded (Everest and Edmundson 1967) on the left side with a unique symbol-orientation combination, and allowed a 1-wk recovery period in the individual aquaria. Pairs were placed in a novel aquarium of the same size as those used in the MIS testing. After 1 h, an observer determined which fish was dominant. The outcome was clear in all pairs, with the dominant fish swimming about the aquarium and the other adopting a submissive posture (Table 1) in a corner of the aquarium or on the bottom. After completion of all paired contests, the fish were killed by an overdose of anesthetic and frozen for future measurement.

Stream tank tests

In 1990, we used the methodology of Fenderson et al. (1968) to establish the dominance ranking for all fish in a group. At the conclusion of the MIS tests, all surviving fish in a group (n = 15 or 16) were transferred into one of the two stream tank sections and allowed to acclimate for 1–3 d. An experimental round began with the observer identifying the dominant fish based on position in the stream section and on the outcome of agonistic interactions. Usually, the dominant fish occupied the upstream half of the section with the other fish arrayed behind in a loose group in the downstream third of the section. Exceptions to this spatial pattern occurred only during interactions between the dominant and subordinate fish. After identifying the dominant fish using the unique brand, the observer recorded the frequency or duration of five behaviours (Table 1) shown by that fish over a 10-min period. In all cases, subsequent interactions confirmed the identity of the dominant fish. The large numbers of fish and the frequency and rapidity of interactions precluded recording behaviours for any but the dominant fish. At the end of the observation period, the observer removed the dominant fish by waiting until it was either alone or in a small group ahead of the partition, dropping the partition, and then netting the dominant fish. This procedure was repeated as many as three times per day over several days until only one fish remained. The round during which a fish was removed is treated here as its dominance ranking (RANK). Note that RANK and dominance ability are inversely related, i.e. the most dominant fish had a RANK of 1.

Morphology

Morphological measurements of the subjects in the paired contest experiments were made after the completion of the experiments. Subjects were thawed, laid on a flat surface, and carefully aligned with fins stretched into lifelike positions. Fork length (FL), body depth (BD, origin of dorsal fin to origin of pelvic fin), caudal fin height (CFH, between posterior tips of the dorsal and ventral lobes of the caudal fin), and the lengths of the leading rays of the anal and dorsal fins (AFIN and DFIN, respectively) were measured with a precision caliper ($\pm$0.1 mm). Measurements were standardized to the mean within-group FL.

Morphological measurements of the subjects in the stream tank experiments were made from the colour photographs made prior to the MIS tests. Photographic images were approximately 1.07× life size. Fork lengths were transformed to within-group normal deviates (mnemonic zFL), allowing us to examine the effects of body length across groups of fish of different sizes. Three measures of body depth were made: BD as above, head depth (HD, truss element 3–4 of Swain and Holtby (1989), and the minimum depth of the caudal peduncle (CP). CFH, AFIN, and DFIN were also measured. Measurements were standardized to the overall mean FL. In both experiments, standardized variables are denoted by a prefixed "s."

Statistical Analysis

Paired contests

Because dominance rank is a binary variable in paired contests, we used logistic regression to test whether morphology and MIS behaviour predict dominance in these contests. Because the data in these tests were for matched pairs of fish, we analyzed these tests using conditional logistic regression for 1–1 matched data (Breslow 1982). Each pair was transformed into a single observation by subtracting the subordinate value from the dominant value for each MIS behaviour and each morphological variable. The transformed data were analyzed using linear logistic models, with the difference variables as explanatory variables, no intercept term, and a constant response variable value (set to zero). Analyses used the SAS LOGISTIC procedure (SAS Institute 1990) with the logit link function. Likelihood ratio tests were used to compare hierarchical models and to test the joint significance of explanatory variables included in models. Significance of individual explanatory variables in multiple regression models was tested using the Wald χ^2 statistic (SAS Institute 1990).

Stream tank tests

Univariate relationships between morphology, MIS behaviours, and dominance rank were examined with Pearson product-moment correlations. Linear models predicting dominance rank from MIS behaviours and morphological variables were calculated using the MGLH procedures of SYSTAT (Wilkinson 1988).

Results

Paired Contests

Morphological and MIS behavioural predictors of dominance

In the trial with newly emerged juveniles, dominance was unrelated to MIS behaviour on the first day of observation but significantly related to MIS behaviour on the fifth day of observation (Table 2). The significance of the model relating dominance to SAM, LAT, and SUB increased progressively from day 1 to day 5. The MIS behaviours on day 5 that predicted dominance most accurately were TOTBEHAV, AGGR, and SAM. Fish with high levels of these behaviours on day 5 tended to be dominant in subsequent paired contests.

In contrast, in the trial with older, experienced juveniles, dominance was significantly related to MIS behaviour on the first day of observation but unrelated to MIS behaviour on later observation days. Fish with high durations of total aggressive behaviour on day 1 ($AGGR_1$) tended to be dominant.

Of the morphological variables examined, BD predicted dominance most closely in the trial with newly emerged juveniles whereas BD and CFH predicted dominance equally well in the trial with older juveniles (Table 3). Individuals with deeper bodies and greater caudal fin heights tended to be dominant. Over the restricted size ranges used in these experiments, BD was more closely related to dominance than were either FL or WT. In both trials, the model predicting dominance from BD was more significant than models in either WT or FL (trial 1: p = 0.004, 0.05, 0.2 respectively; trial 2: p = 0.009, 0.02, 0.08, respectively). In the first trial using newly emerged juveniles, models predicting dominance from FL or WT were significantly improved by including BD as an additional explanatory variable ($\chi^2 \geq 5.628$, df = 1, $p < 0.025$) whereas models predicting dominance from BD were not significantly improved by adding FL or WT ($\chi^2 \leq 1.054$, df = 1, $p > 0.3$). In the second trial using older juveniles, the model predicting dominance from FL was improved by adding BD (χ^2 = 3.837, df = 1, p = 0.05), but models in WT or BD were not significantly improved by adding additional explanatory variables. The size-adjusted variables sBD and sCFH predicted dominance almost as well as the unadjusted variables BD and CFH (Table 3), confirming that effects of BD and CFH were primarily due to shape rather than to general size.

TABLE 2. Significance levels of logistic regression models relating dominance ability in paired contests to prior MIS behaviours. Values of r not significantly different from zero ($p < 0.05$) are italicized. Results are shown for three models: a multivariate model with three predictor variables (SAM, LAT, and SUB) and two univariate models with predictor variables AGGR or TOTBEHAV. Values are probabilities from likelihood ratio χ^2 tests (TOTBEHAV, AGGR, and Model) or from Wald χ^2 tests (SAM, LAT, and SUB). Signs of covariate coefficients are also given.

Observation day	Multivariate model					
	SAM	LAT	SUB	Model	AGGR	TOTBEHAV
			Newly emerged juveniles (n = 32)			
1	−0.8	+0.4	−1.0	0.8	−0.8	−0.8
2	+0.7	+0.4	+0.3	0.4	+0.7	+0.6
3	+0.8	+0.4	+0.3	0.4	−0.9	+1.0
4	+0.2	+0.3	+0.4	0.2	+0.3	+0.2
5	*+0.05*	+0.4	+0.2	*0.02*	*+0.04*	*+0.02*
			7-wk-old juveniles (n = 27)			
1	+0.3	+0.4	−0.1	*0.02*	*+0.03*	+0.1
2	−0.4	+0.8	−0.5	0.4	+0.7	+0.8
3	+0.6	+0.4	−0.5	0.5	+0.2	+0.4
4	+0.6	+0.1	+0.6	0.4	+0.2	+0.1
5	−0.1	−0.5	−0.3	0.1	+0.8	−0.4

TABLE 3. Conditional logistic regression models predicting dominance in paired contests. Units are seconds per 3 min for all MIS behaviours and millimetres for all morphological variables. $\mathcal{L}$ is the logarithm of the likelihood of the model given the data. p gives the joint significance of all explanatory variables included in the model. Subscripts indicate the day of observation.

Model	$-2\mathcal{L}$	P
Newly emerged juveniles (n = 32 (31 for model 4))		
(1) $2.78 \times BD + 0.00713 \times SAM_5 + 0.0994 \times LAT_5 + 0.0693 \times SUB_5$	26.727	0.0015
(2) $2.10 \times BD$	34.707	0.0019
(3) $0.0118 \times SAM_5 + 0.1256 \times LAT_5 + 0.0697 \times SUB_5$	35.047	0.025
(4) $15.005 \times sBD$	36.087	0.0087
(5) $0.0111 \times TOTBEHAV_5$	39.358	0.025
(6) $0.00941 \times AGGR_5$	40.334	0.045
7-wk-old juveniles (n = 27)		
(7) $2.04 \times BD + 0.0227 \times SAM_1 - 0.00658 \times LAT_1 - 0.1182 \times SUB_1$	21.004	0.0025
(8) $1.90 \times BD + 0.0251 \times SAM_1 - 0.1028 \times SUB_1$	21.253	0.0010
(9) $0.0144 \times SAM_1 + 0.00658 \times LAT_1 - 0.0855 \times SUB_1$	27.449	0.019
(10) $0.702 \times CFH$	30.530	0.0087
(11) $1.377 \times BD$	30.623	0.0091
(12) $16.25 \times sBD$	32.161	0.022
(13) $8.76 \times sCFH$	32.486	0.026

For both newly emerged and older juveniles, models including both behavioural and morphological variables were significantly better than models including either variable type alone (Table 3). Among newly emerged juveniles, the model including BD, SAM_5, LAT_5, and SUB_5 was significantly better than the model including just BD ($\chi^2 = 7.980$, df = 3, $p < 0.05$) and the model including just the MIS behaviours ($\chi^2 = 8.320$, df = 1, $p < 0.005$). Similarly, among the older juveniles, the model including BD, SAM_1, LAT_1, and SUB_1 was better than the model with BD only ($\chi^2 = 9.619$, df = 3, $p < 0.025$) and the model with the MIS behaviours only ($\chi^2 = 6.445$, df = 1, $p < 0.02$).

MIS behaviours, stream tank behaviours, and dominance ability

In the stream tank experiments, dominance ability (i.e. a low RANK) was significantly correlated with relatively high levels of SAM behaviour (Table 4; Fig. 1). SLM behaviour, which is superficially similar to SAM behaviour, was only weakly correlated with RANK, although the correlations were in the same direction as for SAM behaviour (Table 4). Dominant fish tended to have shown less MIS LAT_1 and LAT behaviours, but the relationships were not significant in two of three groups (Table 4). LAT_2 and SUB behaviours were not strongly correlated with RANK in any group. The relationships between

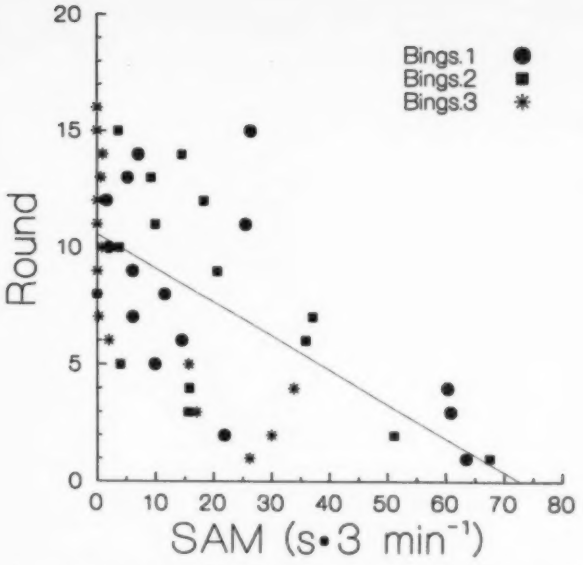

FIG. 1. RANK versus duration of MIS SAM behaviour. The overall least-squares regression line is shown. RANK and SAM are significantly correlated in all groups.

aggressive behaviour (AGGR) and RANK were determined by the relative levels of SAM and LAT behaviours with the result that AGGR was significantly related to RANK in only one of the three groups (Table 4). Total MIS behaviour (TOTBEHAV) was not strongly related to dominance in any group.

Within individual rounds, dominants first interacted with subordinates through approaches followed occasionally by lateral displays but more typically by repeated charges and nips and finally by a chase. The intensity of these interactions between the dominant and the other fish declined significantly with the round (Table 5).

Overt aggressive MIS behaviours (SAM, SLM, OVAGGR) were positively correlated with overt aggressive behaviours in the stream tank but not with the duration of lateral displays in the stream tank (Table 5). MIS LAT behaviours were not strongly correlated with most stream tank behaviours, including lateral displays (Table 5). AGGR, the sum of all aggressive behaviours in MIS, was significantly correlated with all of the recorded stream tank behaviours except NIP and LAT.

Morphological predictors of dominance

Normalized size (zSL) was negatively correlated with RANK in two of the three groups and in the overall comparison (Table 6). Relationships between RANK and measures of body depth were generally weak and inconsistent. The sign of the relationships between RANK and measures of body depth changed from negative to positive with increasing fish size. This change was most pronounced for sBD and sCFH, which were significant predictors of dominance in the paired contest experiments. Dominant fish tended to have relatively long fins (Table 6: all eight correlations were negative). The relationships were strongest in the groups with the largest individuals (Bings.2 and Bings.3).

Multivariate predictors of dominance

We did not use stepwise regression to find the best model from all possible independent variables but instead tested specific models. From univariate correlations we knew that SAM among the MIS behaviours and zSL from the morphological variables were most strongly correlated with RANK, and so we began all modelling with one or both of those variables.

TABLE 4. Pearson product-moment correlations between MIS behaviours and RANK from the stream tank experiments, arranged by population. Mnemonics are detailed in Table 1. Values of r significantly different from zero ($p < 0.05$) are italicized. Note that no measurements were made on the last fish in each trial. Consequently, n is one less than the number of fish in the trial.

	Group			
MIS behaviour	Bings.1	Bings.2	Bings.3	All
SAM	*−0.61*	*−0.62*	*−0.79*	*−0.61*
SLM	−0.08	−0.10	−0.26	−0.09
OVAGGR	−0.45	*−0.56*	*−0.79*	*−0.49*
LAT1	0.51	0.08	0.48	0.21
LAT2	0.09	−0.14	0.05	−0.07
LAT	*0.53*	0.02	0.48	0.18
AGGR	−0.12	−0.50	*−0.52*	−0.25
SUB	−0.04	0.49	0.05	0.21
TOTBEHAV	−0.15	−0.09	−0.37	−0.15
n	14	14	15	43

The best bivariate model (using criterion of explained variance) incorporated both SAM and zSL:

$$\text{RANK} = 9.66 - 0.093 \times \text{SAM} - 2.06 \times \text{zFL}$$

$$(R^2_{\text{adj}} = 0.53, p < 0.001, n = 43)$$

and indicated that large, aggressive fish were dominant. Models incorporating zFL and other measures of MIS aggressive behaviour, such as AGGR or TOTBEHAV, explained less variance but were still highly significant. Dominance ability was greatest in fish that interacted more with their mirror images. In contrast, models incorporating SAM and any of the standardized measures of body depth or fin length were poor predictors of RANK and explained less variance than did the model with SAM alone. Apparently, even the small differences in length present in our groups were important predictors of dominance whereas body depth and fin length appeared to be of little importance.

Discussion

Dominance ability was related to aggressiveness in MIS tests and morphology in all five of our experimental groups. In all groups the best predictors of dominance were SAM, the MIS analog of overt aggressive behaviour, and a morphological variable. The morphological variable most strongly related to dominance ability seemed to vary with age. In the young fish used in the paired contests, body depth was important, and the length or weight of the fish had no additional predictive value (in the situation in which relative length of opponents was controlled). In the older fish used in the stream tank experiments, fish length was most important and body depths were unimportant.

Individual differences in aggressiveness are related to dominance ability. In encounters, with conspecifics, aggressive fish, i.e. those with relatively high levels of certain MIS behaviours, tended to be dominant. Thus, our results extend to the level of the individual what we and others have observed in interpopulation comparisons in coho and chinook salmon (Taylor and

TABLE 5. Pearson product-moment correlations between MIS behaviours or RANK and stream tank behaviours of the dominant fish removed in each round. Values of r significantly different from zero for $n = 43$ ($p < 0.05$) are italicized.

	Stream tank behaviour					
MIS behaviour or RANK	APPROACH	NIP	CHARGE	CHASE	OVAGGR	LAT
SAM	*0.48*	0.15	*0.33*	*0.30*	*0.33*	−0.15
SLM	*0.34*	0.02	0.01	0.04	0.07	0.04
OVAGGR	*0.57*	0.11	0.24	0.24	0.28	−0.07
LAT1	−0.03	0.18	0.22	*0.33*	0.22	0.16
LAT2	0.11	0.19	0.16	*0.32*	0.22	−0.03
LAT	0.02	0.25	0.28	*0.45*	0.30	0.15
AGGR	*0.46*	0.28	*0.41*	*0.54*	*0.46*	0.05
SUB	−0.20	0.07	−0.01	−0.11	−0.04	−0.16
RANK	*−0.48*	*−0.35*	*−0.45*	*−0.40*	*−0.48*	*0.01*

TABLE 6. Pearson product-moment correlations between morphological variables and RANK in the stream tank experiments. Values of r significantly different from zero ($p < 0.05$) are italicized.

		Group			
Morphological variable		Bings.1	Bings.2	Bings.3	All
zFL	Size	*−0.72*	*−0.49*	*−0.81*	*−0.66*
sHD	Body depth	−0.34	0.02	0.02	−0.04
sBD	Body depth	−0.46	−0.15	0.24	−0.03
sCPD	Body depth	−0.18	0.16	−0.04	−0.04
sCFH	Body depth	−0.30	0.10	0.23	−0.04
sAFIN	Fin length	−0.12	*−0.58*	*−0.53*	−0.24
sDFIN	Fin length	−0.24	*−0.60*	−0.20	−0.26
n		15	15	16	46

Larkin 1986; Rosenau and McPhail 1987; Taylor 1988; Swain and Holtby 1989). The explanatory power of MIS measures of individual aggressiveness is only moderate, however. Francis (1990) made a similar observation in an ontogenic (longitudinal) study of behaviour in Midas cichlids. In that study, although aggressive juveniles tended to be dominant, the explanatory power of MIS measures of aggressiveness was low and declined as the fish aged. Francis began his study with 10-wk old individuals that were already socially experienced, so the initial relationship between individual aggressiveness and dominance is unknown. Nevertheless, other factors besides aggressiveness have some role in determining dominance ability.

MIS behaviour of recently emerged juveniles predicted subsequent dominance in paired interactions on the fifth but not on earlier observation days. Agonistic behaviour appears to develop over a period lasting several days in coho salmon isolated soon after emergence (Rosenau 1984; Rosenau and McPhail 1987; Swain and Riddell 1990). This developmental period may explain the failure of MIS behaviour on early observation days to predict dominance using recently emerged juveniles. A developmental period is not evident in the MIS behaviour of experienced juveniles (e.g. Swain and Holtby 1989), and MIS behaviour on the first observation day was the best predictor of dominance in paired contests using socially experienced juveniles in this study.

The failure of MIS behaviour on the second to fifth observation days to predict dominance in paired contests between experienced laboratory-reared juveniles in our study is unexplained. A similar effect was not observed in our stream tank tests using experienced wild-reared juveniles. This difference between paired contests and stream tank tests could reflect either the differences in test conditions or the differences in rearing environments between the two experiments and requires further study.

The significance of MIS lateral display behaviours appears to differ fundamentally between within-population and between-population comparisons. In our comparisons of individuals within populations, MIS display behaviours were not generally related to either dominance ability or to levels of agonistic behaviour in stream tank tests. In contrast, in comparisons between populations, differences in mean MIS LAT duration do parallel differences in aggressiveness between populations as characterized by either dominance ability in paired contests or levels of aggressive behaviours in stream tanks (e.g. Rosenau and McPhail 1987; Taylor 1988). Lateral displays have been interpreted as arising from a conflict between escape and attack motivations (Baerends and Baerends-van Roon 1950; Barlow 1962; Keenleyside and Yamamoto 1962; Rasa 1969). Lateral displays are used by Atlantic salmon (*Salmo salar*) at territory boundaries in interactions between neighbours rather than betweeen territory holders and intruders (Keenleyside and Yamamoto 1962). Territory holders repel intruders with overt aggressive acts, such as charges, chases, and nips. Thus, in MIS tests, LAT may indicate a lower level of aggressiveness than SAM. Because individual coho appear to use overt aggressive behaviours rather than display behaviours to establish and maintain dominance, it is not surprising that the MIS analog of overt aggressive behaviour (i.e. SAM) is the better predictor of dominance ability in comparisons among individuals. Dif-

ferences between populations in the extent of lateral display behaviours may reflect differences in the level of involvement in social interaction rather than differences in aggressiveness. In populations with a high level of involvement in social interaction, rates of lateral display and overt aggressive behaviour may both be high. In this case, rates of lateral display and aggressiveness will be positively correlated at the population level even though they are not at the level of individuals within populations.

Body depth and median fin size may influence apparent size during lateral displays. Lateral displays were not related to dominance ability either in paired contests or in the stream tanks. Consequently, our failure to demonstrate any consistent relationship between median fin size and dominance is not surprising. However, body depth was generally related to dominance in the predicted direction in our experiments. This relationship was stronger in the smaller and younger fish used in the paired contests than in the stream tank experiments. In the paired contests, deeper bodied fish were significantly more likely to be dominant in both trials. In the stream tank experiments, deeper bodied fish tended to be dominant, but in no case was this relationship statistically significant. We suggest that body depth and dominance ability are correlated because of the effect of body depth on strength (i.e. burst swimming performance (Webb 1977, 1978; Taylor and McPhail 1985)) rather than because of the effect of body depth on lateral profile during display behaviours.

Francis (1990) found that size and dominance were closely related in Midas cichlids, but it was unclear whether size determined dominance ability or was a consequence of it. In coho salmon, dominance relationships established on the basis of size differences cannot be reversed through size reversals (Abbott and Dill 1989). In our paired-contest experiments, fish size (FL or WT) was not associated with dominance ability, but dominance ability was associated with individual aggressiveness and lateral body profile. This leads us to speculate that size may be more a consequence of dominance status than a cause of it in coho salmon. Thus, the significant positive relationship between body size and dominance that we observed in older fish might have been a consequence of aggressive fish growing faster, probably through differential access to food (Yamagishi 1962; Wańkowski and Thorpe 1979; Metcalfe et al. 1988; Abbott and Dill 1989). Resolution of this question awaits detailed longitudinal studies beginning with newly emerged, socially naive fry (Huntingford et al. 1990).

We have demonstrated that individual differences in aggressiveness and morphology are both related to dominance ability. Given the possibility that current hatchery practices are leading to both increased aggressiveness in, and morphological divergence of, hatchery stocks (Flick and Webster 1964; Moyle 1969; Reisenbichler and McIntyre 1977; Ryman and Stahl 1980; Hjort and Schreck 1982; Bachman 1984; Taylor 1986; Fleming and Gross 1989; Swain and Riddell1990; Swain et al. 1991), the straying of hatchery fish into wild populations presents a real and potentially serious threat to the long-term productivity of our remaining wild coho populations.

Acknowledgements

We gratefully acknowledge the assistance of S. Baillie, D. Davies, B. Finnegan, and K. Simpson in collecting and maintaining the fish used in our study. We thank Dr. Neil B. Metcalfe and an anonymous reviewer for helpful critical reviews. Funding was provided by the Department of Fisheries and Oceans in support of the International Pacific Salmon Treaty.

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ANNOUNCEMENTS/AVIS

European Marine Biology Symposium (EMBS)

The European Marine Biology Symposium (EMBS) is organised each year in a different European country. Details for the next three years are given below.

28th EMBS

23–28 September 1993, in Crete, Greece; contact Mrs. V. Dessipri, Institute of Marine Biology of Crete, P.O. Box 2214, Heraklion 71003, Crete. Topic: Biology and ecology of shallow coastal waters.

29th EMBS

28 August – 2 September 1994, in Vienna, Austria; contact Professor J. Ott, Institute of Zoology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria. Topics: (1) Influence of organisms on their environment and (2) Role of episodic events.

30th EMBS

11–15 September 1995, in Southampton, England; contact Dr. P. A. Tyler, Department of Oceanography, University of Southampton, Southampton SO9 5NH, England. Topic: Environmental control of physiological and behavioural processes in marine organisms.

Symposium européen de biologie marine (EMBS)

Chaque année, un pays européen différent est l'hôte du Symposium européen de biologie marine (EMBS). Au cours des trois prochaines années, ce symposium aura lieu dans les villes suivantes :

28e EMBS

Du 23 au 28 septembre 1993, à Crète, en Grèce; personne-ressource : Mme Dessipri, Institut de biologie marine de Crète, B.P. 2214, Heraklion 71003, Crète. Sujet : La biologie et l'écologie des eaux côtières peu profondes.

29e EMBS

Du 28 août au 2 septembre 1994, à Vienne, en Autriche; personne-ressource : Professeur J. Ott, Institut de zoologie, Université de Vienne, Althanstr. 14, A-1090 Vienne, Autriche. Sujets : 1) L'incidence des organismes sur leur environnement et 2) Le rôle des événements épisodiques.

30e EMBS

Du 11 au 15 septembre 1995, à Southampton, Angleterre; personne-ressource : P. A. Tyler, Department of Oceanography, University of Southampton, Southampton SO9 5NH, England. Sujet : Le contrôle de l'environnement des processus physiologiques et comportementaux chez les organismes marins.

Aquatic Toxicity Workshop

The twentieth in a series of Annual Aquatic Toxicity Workshops will be held on October 17–20, 1993, at the Chateau Frontenac, Quebec City, Quebec. Major topics proposed for this Workshop are Sources of Toxicity, Pathways and Fate of Toxicants, Toxicokinetic Aspects, Toxicodynamic Mechanisms, Toxicity Testing Methods and QA/QC, Genotoxicity, Food Chain Modelling, Exposure and Risk, in situ Bioindicators, Microcosms and Mesocosms, Bioprobes, Environmental Effects Monitoring, Aquatic Toxicology and Human Toxicology, and Aquatic Toxicity Regulations.

Other activities planned include a special conference on the Saint Lawrence Action Plan (1988–93), an excursion on the Saint Lawrence River Estuary to view beluga whales, and tours of the Maurice Lamontagne Institute, which is a Department of Fisheries and Oceans research facility in Mont Joli, and the Oceanographic Center of the University of Quebec in Rimouski.

Platform and poster presentations will be accepted in English or French, and submissions must be received by **September 11, 1993**, to be considered. For further information, please contact Dr. Raymond Van Coillie, Organizing Committee Chair, Environment Canada, C-P., Quebec Region, 1179 de Bleury Street, Montreal, Quebec H3B 3H9, telephone (514) 496-6858, Fax (514) 283-4423, or Dr. Peter Campbell, Scientific Subcommittee Chair, University of Quebec, INRS-Eau, 2700 Einstein Street, Ste-Foy, Quebec G1V 4C7, telephone (418) 654-2538, Fax (418) 654-2562.

Atelier annuel sur la toxicité aquatique

Le vingtième d'une série d'Atelier annuel de la toxicité aquatique aura lieu du 17 au 20 octobre 1993 au Château Frontenac, Québec. Les sujets de discussion d'importance majeure de cet atelier sont : Sources de toxicité, Cheminements et sorts des toxiques, Aspects toxicocinétiques, Mécanismes toxicodynamiques, Bioessais de toxicité et leur QA/QC, Génotoxicité, Chaîne trophique, Exposition et risque, Bioindicateurs in situ, Microcosmes and mésocosmes, Biomarqueurs, Suivi des effets environnementaux, Toxicologie aquatique et toxicologie humaine et Toxicologie aquatique et réglementation.

Autres activités planifiées incluent une Conférence spéciale sur le Plan d'Action Saint Laurent (1988–1993), une excursion sur l'estuarie du Saint Laurent pour régarder les baleines bélugas et une Tournée de l'Institut Maurice-Lamontagne, attribué au ministère des Pêches et des Océans, une facilité de recherche à Mont-Joli, et le Centre Océanographique de l'Université de Québec à Rimouski.

Les présentations, sous forme d'exposés ou d'affiches, peuvent être soumises en anglais ou en français et doivent être reçues au plus tard de **11 septembre 1993**. Des renseignements supplémentaries peuvent être obtenus du Dr. Raymond Van Coillie, Président du comité organisateur, Environnement Canada, C-P., Région du Québec, 1179, rue de Bleury, Montréal (Québec) H3B 3H9, téléphone : (514) 496-6858, télécopieur : (514) 283-4423, ou Dr. Peter Campbell, Président du sous-comité scientifique, Université du Québec, INRS-Eau, 2700, rue Einstein, Ste-Foy (Québec) G1V 4C7, téléphone : (418) 654-2538, télécopieur : (418) 654-2562.

Second International Symposium on Abalone Biology, Fisheries, and Culture

The Symposium will be held 7–11 February 1994 at the University Centre, University of Tasmania. For further information, contact Warwick Nash, Sea Fisheries Division, Crayfish Point, Taroona, TAS 7053, Australia. Telephone: +61 02 277277; facsimile: +61 02 278035.

Deuxième Symposium international sur la biologie, les pêcheries et la culture de l'ormeau

Le Symposium aura lieu du 7 au 11 février 1994 au University Centre, University of Tasmania. Pour renseignements, contacter Warwick Nash, Sea Fisheries Division, Crayfish Point, Taroona, TAS 7053, Australia. Téléphone : +61 02 277277; télécopieur : +61 02 278035.

Canadian Conference for Fisheries Research (CCFFR)

Delta Bessborough Hotel
Saskatoon, Saskatchewan
January 4–5, 1994

Announcement of Conference and Call for Papers

This conference will be held in Saskatoon, Saskatchewan, January 4 and 5, 1994. The major themes of the conference will be (1) Arctic and Northern Fisheries Biology, (2) Aquatic Habitat Manipulation, and (3) Novel Approaches to Fish Bioenergetics.

Papers on the major themes are particularly encouraged but submissions on other themes related to Fisheries and Habitat Biology are also welcome.

Deadline for abstracts is **August 30, 1993**. They should be typed (maximum 200 words, single spaced) and nature of presentation (oral/poster) indicated. A high quality of copy is required, suitable for photographic reproduction.

Abstracts should be addressed to Dr. R. J. F. Smith, Head, Biology Department, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada. Telephone: (306) 966-4400.

Conférence canadienne sur recherche sur les pêches (CCRP)

Delta Bessborough Hotel
Saskatoon (Saskatchewan)
les 4 et 5 janvier 1994

Avis de conférence et invitation à présenter des communications

La conférence aura lieu les 4 et 5 janvier 1994 à Saskatoon, Saskatchewan. Les principaux thèmes abordés seront les suivants : 1) Biologie des pêches de l'Arctique et du nord, 2) Remaniement de l'habitat aquatique et 3) Nouvelles approches pour aborder la bioénergétique chez les poissons.

Nous sollicitons particulièrement les présentations sur les thèmes susmentionnés, mais nous acceptons également les communications sur d'autres thèmes reliés à la recherche halieutique et à la biologie des poissons.

La date limite pour l'envoi des résumés a été fixée au **30 août 1993**. Les résumés doivent être dactylographiés (200 mots au maximum, à simple interligne). Il est important de préciser sous quelle forme se déroulera la présentation (présentation orale/affiche). Une copie de très bonne qualité est requise aux fins de reproduction photographique.

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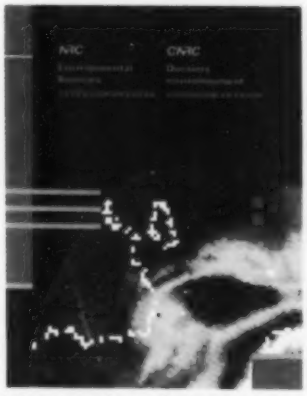

The National Research Council of Canada is pleased to announce that the first issue of Environmental Reviews will appear early in 1993. This review journal in the environmental sciences is a welcome complement to the 12 primary research journals already published by NRC in a variety of science and engineering fields. Articles, international in outlook yet with a significant Canadian contribution, are expected to be very readable and understandable to a broad range of scientists and environmentalists. Contributions, whether invited or unsolicited, will be peer reviewed by a panel of associate editors drawn from universities and research institutes around the world.

Published papers will normally be 8-14 printed pages in length, although may go to 25 pages in exceptional circumstances. Papers are sought on a broad range of topics including climate change, environmental impact of forestry practice, crop responses to air pollution, methane budgets and sources, erosion, pesticide resistance, mega-project impacts, invasions of alien species, reclamation, heavy metals, PCBs, and a host of others.

Authors interested in submitting manuscripts should contact:
Dr. Thomas C. Hutchinson, Environmental Reviews, Environmental Sciences Centre, Trent University, Peterborough, Ont., Canada K9J 7B8 (Telephone/FAX: (705) 748-1634)

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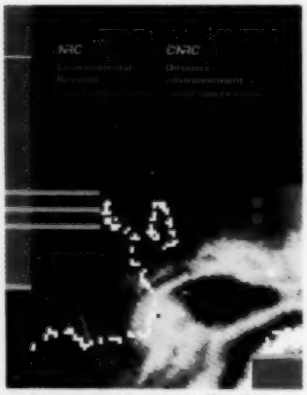

Le Conseil national de recherches du Canada a le plaisir d'annoncer que le premier numéro des Dossiers environnement sera publié au début de 1993. Cette publication sur les sciences de l'environnement est un complément apprécié aux 12 revues de recherche primaire publiées par le CNRC dans une diversité de domaines scientifiques et techniques. Les articles qui y seront publiés, tout en ayant une envergure internationale, bénéficieront d'un importante contribution canadienne et leur contenu devra être accessible à la majorité des scientifiques et des environnementalistes. Les contributions, sollicitées comme non sollicitées, seront examinées par un comité de directeurs scientifiques associés provenant d'universités et d'instituts de recherche du monde entier.

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Journal canadien des sciences halieutiques et aquatiques

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Parsons, G. J., S. M. C. Robinson, J. C. Roff, and M. J. Dadswell. Daily growth rates as indicated by valve ridges in postlarval giant scallop (*Placopecten magellanicus*) (Bivalvia: Pectinidae) 456–464
Hoogenboezem, W., E. H. R. R. Lammens, P. J. MacGillavry, and F. A. Sibbing. Prey retention and sieve adjustment in filter-feeding bream (*Abramis brama*) (Cyprinidae) 465–471
Hoogenboezem, W., and J. G. M. van den Boogaart. Importance of mucus in filter-feeding of bream (*Abramis brama*) 472–479
Johnsson, J. I., W. C. Clarke, and R. E. Withler. Hybridization with domesticated rainbow trout (*Oncorhynchus mykiss*) reduces seasonal variation in growth of steelhead trout (*O. mykiss*) 480–487
Chauvet, E., N. Giani, and M. O. Gessner. Breakdown and invertebrate colonization of leaf litter in two contrasting streams: significance of oligochaetes in a large river 488–495
Aalto, S. K., and G. E. Newsome. Winds and the demic structure of a population of yellow perch (*Perca flavescens*) 496–501
Foster, A. R., D. F. Houlihan, and S. J. Hall. Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod (*Gadus morhua*): comparison of morphological and biochemical measurements 502–512
Shardlow, T. F. Components analysis of a density-dependent catchability coefficient in a salmon hook and line fishery 513–520
Parkman, H., and M. Meili. Mercury in macroinvertebrates from Swedish forest lakes: influence of lake type, habitat, life cycle, and food quality 521–534
Dorazio, R. M. Prerelease stratification in tag-recovery models with time dependence 535–541
Martoja, M. et C. Marcaillou. Localisation cytologique du cuivre et de quelques autres métaux dans la glande digestive de la seiche, *Sepia officinalis* L. (Mollusque Céphalopode) 542–550
Neilson, J. D., J. F. Kearney, P. Perley, and H. Sampson. Reproductive biology of Atlantic halibut (*Hippoglossus hippoglossus*) in Canadian waters 551–563
McGarvey, R., F. M. Serchuk, and I. A. McLaren. Spatial and parent-age analysis of stock–recruitment in the Georges Bank sea scallop (*Placopecten magellanicus*) population 564–574
Bulger, A. J., L. Lein, B. J. Cosby, and A. Henriksen. Brown trout (*Salmo trutta*) status and chemistry from the Norwegian Thousand Lake Survey: statistical analysis 575–585
Martens, D. W., and J. A. Servizi. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.) 586–590
Whyte, J. N. C., R. J. Beamish, N. G. Ginther, and C.-E. Neville. Nutritional condition of the Pacific lamprey (*Lampetra tridentata*) deprived of food for periods of up to two years 591–599
Koenings, J. P., H. J. Geiger, and J. J. Hasbrouck. Smolt-to-adult survival patterns of sockeye salmon (*Oncorhynchus nerka*): effects of smolt length and geographic latitude when entering the sea 600–611
Hannah, R. W. Influence of environmental variation and spawning stock levels on recruitment of ocean shrimp (*Pandalus jordani*) 612–622
Lamontagne, S., and J. B. Rasmussen. Estimating crayfish density in lakes using quadrats: maximizing precision and efficiency 623–626
Smith, E. P., D. R. Orvos, and J. Cairns, Jr. Impact assessment using the before-after-control-impact (BACI) model: concerns and comments 627–637
Rodríguez, M. A., P. Magnan, and S. Lacasse. Fish species composition and lake abiotic variables in relation to the abundance and size structure of cladoceran zooplankton 638–647
Ashton, H. J., D. O. Farkvam, and B. E. March. Fatty acid composition of lipids in the eggs and alevins from wild and cultured chinook salmon (*Oncorhynchus tshawytscha*) 648–655
McCormick, S. D. Methods for nonlethal gill biopsy and measurements of Na^+,K^+-ATPase activity 656–658
Guénette, S., R. Fortin, and E. Rassart. Mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River and James Bay drainage basins in Quebec, Canada 659–664
Triska, F. J., C. M. Pringle, G. W. Zellweger, J. H. Duff, and R. J. Avanzino. Dissolved inorganic nitrogen composition, transformation, retention, and transport in naturally phosphate-rich and phosphate-poor tropical streams 665–675
Holtby, L. B., D. P. Swain, and G. M. Allan. Mirror-elicited agonistic behaviour and body morphology as predictors of dominance status in juvenile coho salmon (*Oncorhynchus kisutch*) 676–684

Announcements/Avis 685–686

CJFSDX50(3) 455–686 (1993)
ISSN 0706-652X

Canada

Typeset and printed in Canada by/
Compose et imprime au Canada par
Lowe-Martin Group: Runge Press Division

0706-652X(199303)50:3;1-O